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Virus Diseases.

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Studies on the Neurotropic Virus of Horsesickness. VI.—Propagation in the Developing Chick Embryo.

By R. A. ALEXANDER, Section of Virus Diseases, Onderstepoort.

THE introduction into general practice of the neurotropic virus method of immunization of horses and mules against horsesickness has necessitated the annual production of sufficient virus for the treatment of approximately 40,000 animals. The entire supply of virus has been obtained by propagating it in the brains of mice by the methods detailed in a previous publication (Alexander, Neitz and du Toit 1937). The present vaccine contains 5 antigenically different strains of virus, each of which is propagated separately and two of which are present only in low titre in the brains of moribund mice. Bearing this in mind, together with the fact that large numbers of mice are required for research into many essential problems connected with immunity and vaccine production, it is apparent that any interference with the regular supply of mice is a most serious matter. Consequently, apart from the scientific value attached to the discovery of a number of other methods for the production of large supplies of virus, a substitute for mice has become of extreme practical importance.

Initially, it was believed that the susceptible guinea pig with its larger central nervous system would be the animal of choice, but when it was found that an emulsion prepared from a single guinea pig brain invariably contained no more virus than an equal volume of emulsion prepared from one mouse brain, the use of guinea-pigs was abandoned. Similar observations were made in rats; rabbits, being insusceptible to the virus, were of no value whatever.

A cursory investigation of the possibility of using the recognized tissue culture method of virus cultivation namely, cultivation in an appropriate modification of the Maitland medium was carried out. It was apparent at an early stage that, in spite of definite though somewhat irregular multiplication, the method was of no value for the production of a high titre virus emulsion.

Finally, the work of Woodruff and Goodpasture (1931) and Goodpasture and his collaborators (1932) with vaccinia prompted the use of the developing chick embryo or its membranes as a suitable culture medium, more particularly because a number of investigators has used this technique not only for the propagation of many different viruses, but also have adapted modifications to the study of problems such as immunity production and virus identification. The results of the cultivation of neurotropic horsesickness virus in the developing chick embryo form the basis of this communication.

No attempt is made to review the relevant literature on this subject since this has been dealt with adequately in recent publications by Burnet (1936).

Technique.—A slight modification of Burnet's (1936) technique is employed with the object of simplifying the procedure as far as possible with an eye to mass production of virus.

Eggs are obtained from mated pens of young hens maintained at the Institute. An ordinary electrically controlled chicken incubator running at 103° F. (39·4° C.) is used for incubation, and before the fresh eggs are added every day no precautions were taken other than to wash them carefully but thoroughly in clean water. On the third or fourth day of incubation the eggs are candled in the usual way and all " infertiles " and " dead-in-shells " discarded. For seeding purposes eggs containing 8-9 day old embryos are used. If younger embryos are used there is a danger of damaging the poorly developed vascular membranes during the manipulations, resulting in a high percentage of early deaths of the embryos; alternately, if older embryos are used the fluff and feathers become a source of great inconvenience when harvesting the virus.

The selected egg is washed with 96 per cent. alcohol and the position of the air space, the embryo and the membranes noted by transillumination. A 250 candle power " Pointolite " lamp in a dark room is well suited to this purpose, and it was soon apparent that the entire operation of seeding may be carried out most conveniently in the beam from this lamp. By means of a dental drill* fitted with a No. 2 Solila burr a hole ±0·5 mm. in diameter) is bored through the shell and outer shell membrane into the air space. It is advisable to drill this hole first as it gives the operator a good idea of the density and thickness of the egg shell, properties which were found to vary within very wide limits. A second hole of the same size is then drilled at a point about the centre of the long axis of the egg, over the position of maximum development of the chorio-allantoic membrane. This hole must penetrate the shell only and should leave the shell membrane intact. With the point of a fine sterile needle the exposed shell membrane is pricked and a minute triangular flap detached from the underlying vascular membrane using a picking rather than a piercing movement. The air is evacuated from the air space by means of a rubber teat. This results in the formation of an artificial air space usually about 2-2·5 cm. in diameter by 3 mm. in greatest depth bounded below by the chorio-allantoic membrane and above by the shell membrane which remains in apposition to the pierced shell. The point of a fine needle fitted to a syringe is inserted into the artificial air space and the inoculum ejected on to the membrane. Both holes are sealed with melted paraffin wax, and the egg is placed in an incubator at the required temperature.

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* A dental drill was constructed locally from an ordinary Singer sewing machine motor with variable speed foot control, the dental handpiece being attached to a 3-ft. length of motor-car speedometer flexible drive which in turn was welded on to the motor spindle.

For optimum results certain precautions are necessary. Care should be taken to drill the hole, through which the inoculation is to be carried out, at a point between the anastomotic branches of the bloodvessels and not directly over a large vessel which might be punctured and cause considerable haemorrhage. Should the chorio-allantoic membrane be pierced, an artificial air space will not result from evacuation of the true air space, but a bubble will be formed within the albumen and naturally the inoculum will not be deposited upon the membrane. This may be controlled by observing by trans-illumination that during evacuation a widening shadow is formed starting from the point of entry of air and that simultaneously the clearly visible blood vessels disappear over this area from their position beneath the shell. An air bubble within the albumen is recognized by being easily movable and by retaining its dorsal position on rotating the egg. All the air must be sucked out of the air space so as to bring the outer and inner shell membrane together or else it will be found that, after a few days incubation, there is a tendency for the artificial air space to disappear wholly or partially, and for the true air space to reform; such an occurrence greatly hampers the subsequent removal of the membrane in a sterile manner. During incubation the eggs should be immobilized by placing them upon a wire screen of suitable mesh or upon cotton wool so as to prevent rolling and the detachment of too large a portion of foetal membrane from the egg shell. Throughout the operation reasonable aseptic precautions only are necessary. For instance it has been found adequate to clean and disinfect the dental burr each time merely by dipping it into alcohol, and no advantage was gained from flaming the eggs or washing them in strong disinfectant.

The above described operation may sound somewhat tedious, but actually this is not the case. In actual practice a technician with average " hands " will be able to drill down to the shell membrane by sense of feel alone with great rapidity and, after a little experience will be able to seed and seal off eggs at the rate of approximately one per minute with an embryo mortality rate of not more than 5 per cent.

When it is desired to harvest the membrane or embryo the shell in the neighbourhood of the point of inoculation is cracked by tapping with a blunt instrument. The shell is detached carefully with forceps leaving the shell membrane exposed and practically intact over an area which will depend in size upon the age and development of the embryo. The shell membrane is torn off, the chorio-allantoic membrane grasped with forceps and the requisite portion snipped off with the scissors. The embryo is grasped by the neck with forceps taking care not to rupture the yolk sac, lifted out and the umbilical vessels severed. Aseptic precautions are used throughout.

In the present study the volume of inoculum was 0·15 c.c of a 5 per cent. emulsion of the infective material in 10 per cent. normal horse serum-saline. Incubation was carried out at 37·5° C. In a few experiments an incubation temperature of 32° C. was tried without any apparent advantage; no work was done at temperatures higher than 37·5° C. In the great majority of cases sub-inoculation was carried out on the fourth day after seeding.

As embryo brain, is considered that portion of the head which remains after snipping off the beak, and enucleating the eyes; the term brain therefore is not strictly accurate but the arbitrary procedure obviated the necessity of removing the soft, pulpy brain from the relatively small amount of cartilaginous skull. Emulsions are made by mincing the material as finely as possible with scissors and grinding it up with a roughened glass rod without sand before adding the diluent. Then the emulsion is forcibly sucked into and ejected from a syringe until the solid particles are finely disintegrated. In the later experiments emulsions were frozen and thawed rapidly to facilitate disintegration, a procedure which appeared to increase the titre slightly. The supernatant fluid obtained after centrifugation at 3,000 revs. per min. for 15 minutes is titrated by the injection of decimal dilutions in 0·05 c.c. amounts intracerebrally into mice and the figures given represent dilutions of the original infective material.

The strain of virus used was that known as 20449 which was in its 181st intracerebral passage in mice. No work has been carried out with other virus strains.

After a few preliminary trials it was apparent that the greatest multiplication of virus took place in the brain of the embryo. It was decided to passage the virus in series through eggs for several generations and then to determine the site and rate of multiplication. The results obtained through 17 passages are shown in tabular form in Table 1.

A consideration of the results brings out the following points:—

1. The virus was maintained by serial passage through the brains of chick embryos for 17 generations when the experiment was discontinued voluntarily.

2. Undoubted multiplication of virus occurred. Had no multiplication taken place the titre in successive generations would have decreased progressively so that the infectivity for mice would certainly have been lost by the fifth generation.

3. The virus titre attained on the fourth day was fairly constant at a level only slightly below that of the original mouse brain emulsion.

4. In one instance only (egg 1, generation 8) was no virus detectable in the embryo on the fourth day. The reason is quite obscure but it probably represents a single instance of faulty technique of inoculation.

Multiplication of Neurotropic Virus in the Brain of Chick Embryos.

Generation.	Date of Seeding.	Date of Harvesting.	Time of incubation days.	Egg No.	VIRUS TITRATION.				
					1/20.	1/100	1/1,000.	1/10,000.	1/100,000.
—	—	—	—	—	‡ 3,4	5,6	6,7	7,0	00
1	10.3.36	13.3.36	3	{1	4,5	4,0	5,5	0,0	x 0
				2	4,5	5,6	6,0	0,0	x 0
		14.3.36	4	*{3	x 3	4,5	3,5	5,6	5,6
				4	4,5	4,6	3,4	0,0	3,0
		15.3.36	5	5	4,4	4,5	5,0	0,0	0,0
2	14.3.36	18.3.36	4	*{1	5,5	5,5	5,0	0,0	0,0
				2	5,5	5,5	5,0	x 3	0,0
		19.3.36	5	3	4,4	5,6	5,0	6,0	0,0
3	19.3.36	23.3.36	4	*{1	7,0	6,6	0,0	0,0	0,0
				2	5,5	5,0	5,5	7,0	0,0
4	23.3.36	26.3.36	3	1	4,4	4,5	4,4	0,0	0,0
		27.3.36	4	*{2	5,5	3,4	5,5	5,5	0,0
				3	3,4	4,5	5,0	3,0	0,0
		28.3.36	5	4	4,4	4,4	4,0	4,0	0,0
5	27.3.36	31.3.36	4	*{1	4,5	5,6	0,0	0,0	0,0
				2	5,5	5,6	0,0	0,0	0,0
6	31.3.36	4.4.36	4	*{1	4,4	4,4	5,5	6,0	0,0
				2	4,4	4,5	5,5	6,0	0,0
7	4.4.36	8.4.36	4	*{1	5,5	5,5	5,0	6,0	x 0
				2	5,0	5,5	6,0	5,0	0,0
8	10.4.36	14.4.36	4	*{1	0,0	0,0	0,0	0,0	0,0
				2	6,6	6,6	6,0	0,0	0,0
9	16.4.36	20.4.36	4	*{1	5,5	5,6	7,0	0,0	0,0
				2	5,0	5,5	5,5	0,0	0,0
10	8.6.36	9.6.36	1	1	0,0	0,0	0,0	0,0	0,0
		10.6.36	2	2	0,0	0,0	0,0	0,0	0,0
		11·6·36	3	*3	5,5	5,5	0,0	—	—
11	12.6.36	15.6.36	3	1	4,6	5,7	6,6	—	—
		16.6.36	4	*2	5,0	5,5	4,6	—	—
		17.6.36	5	3	4,5	4,7	0,0	6,0	—
		18.6.36	6	4	4,4	5,5	0,0	0,0	—
12	16.6.36	20.6.36	4	*{1	4,4	4,4	5,6	4,5	—
				2	4,5	x 5	5,5	0,0	—
13	22.6.36	26.6.36	4	*{1	5,5	5,0	5,0	0,0	—
				2	4,4	4,5	5,0	0,0	—
14	26.6.36	29.6.36	3	1	5,6	6,6	6,6	6,0	0,0
		30.6.36	4	2	5,6	5,0	0,0	x 0	0,0
		1.7.36	5	*3	4,5	5,5	5,5	6,0	6,0
15	6.7.36	10.7.36	4	*{1	4,5	5,5	5,7	7,0	0,0
				2	4,5	4,4	4,5	7,0	0,0
16	10.7.36	14.7.36	4	1	5,5	5,5	5,5	0,0	—
[illegible]	[illegible]	[illegible]	5	1	4.7	4.6	5,6	0,0	0,0

Concurrently with this experiment an attempt was made to determine the site of greatest multiplication of virus, and the optimum time of incubation for maximum yield. Embryos were divided into three portions:—

(*a*) Membrane—a portion of the chorio-allantoic membrane usually about 2 cm. square selected from the site of inoculation.

(*b*) Head—as before.

(*c*) Body—the entire embryo minus the head.

Details of the experiments together with the results are given in Table II. In each case a 5 per cent. emulsion was made as before and dilutions are in terms of the material harvested.

Results.

A consideration of the results brings out the following points:—

1. *Membrane.*—It is not possible to determine whether multiplication of the virus took place on the chorio-allantoic membrane since the virus demonstrated may represent simply survival of the inoculum; if multiplication did take place it was slight. From the twenty-fourth hour there was a steady decrease in the titre of the membrane so that from the third to the eighth day of incubation no more than a trace could be demonstrated.

2. Body.—In the body, twenty-four hours after seeding, no virus was detectable. Virus had made its appearance by the forty-eighth hour, and persisted, if only in traces, up to the seventh day. It is worthy of note that as the virus content of the membrane decreased so did that of the body rise, but the titre was always low. This suggests that the virus demonstrated at the forty-eighth hour represented that which had been absorbed from the membrane, that little or no multiplication took place in the body outside the spinal cord and nerves which were not separated from the parenchymatous organs or the skin.

3. *Head.*—The site of the greatest multiplication was in the brain of the embryo. Virus had made its appearance by the forty-eighth hour in greater concentration than in either the membrane or body. The titre increased rapidly to reach a maximum on the fourth to fifth day after which there was a gradual but steady decline until only traces were demonstrable on the eighth day of incubation.

The Effect of the Virus on the Embryo.

At no time was any gross pathological lesion on the membrane observed; usually there was noticed slight oedema and opacity particularly at the twenty-fourth hour but these changes were never more marked than in control eggs which had been seeded with normal saline. The formation of discrete pocks or opaque areas was never observed. The parenchymatous organs appeared normal. In the brain, particularly on the fourth and fifth day, slight haemorrhagic infiltration in the form of minute red dots was noted.

TABLE II.

Virus content of Membrane, Body and Head of Chick Embryos.

Time of Incubation.	Virus Titration.										
	Membrane.			Body.			Head.				
	1/20.	1/100.	1/1,000.	1/20.	1/100.	1/1,000.	1/20.	1/100.	1/1,000.	1/10,000.	1/100.000.
24 hours.........	5,5 5,0 5,5	6,0 5,0 6,7	0,0 0,0 0,0	0,0 0,0 0,0	0,0 0,0 x 0	0,0 0,0 0,0	0,0 0,0 0,0	0,0 0,0 0,0	0,0 0,0 0,0	— — —	— — —
48 hours.........	0,0 4,6 6,6	0,0 0,0 6,6	0,0 0,0 0,0	5,6 4,5 5,0	x 0 5,0 0,0	0,0 6,0 0,0	0,0 5,5 6,6	0,0 5,5 6,6	0,0 5,5 x 0	— — —	— — —
3 days..........	8,0 0,0 0,0 0,0 6,0	0,0 0,0 0,0 0,0 0,0	0,0 0,0 0,0 0,0 0,0	5,0 5,6 6,6 0,0 6,7	5,0 0,0 0,0 x 0 7,0	0,0 0,0 0,0 0,0 0,0	5,5 4,6 5,6 4,5 5,7	5,5 5,7 6,6 4,5 6,0	0,0 6,6 6,6 0,0 0,0	— — 6,0 0,0 0,0	— — 0,0 0,0 0,0
4 days..........	4,0 0,0 0,0 0,0	0,0 0,0 0,0 0,0	0,0 — 0,0 0,0	x 0 0,0 0,0 7,7	0,0 0,0 0,0 0,0	0,0 — 0,0 0,0	5,0 5,6 4,5 4,5	5,5 5,0 4,4 5,5	4,6 0,0 4,5 5,7	— x 0 7,0 7,0	— 0,0 0,0 0,0
5 days..........	0,0 0,0 0,0	0,0 0,0 0,0	— — —	6,0 6,0 0,0	0,0 0,0 0,0	— — 0,0	4,5 4,5 4,7	4,7 4,5 4,6	0,0 5,5 5,6	6,0 6,0 0,0	— 6,0 0,0
6 days..........	0,0 0,0	0,0 0,0	— 0,0	5,0 6,7	0,0 6,0	— 0,0	4,4 6,5	5,5 6,6	0,0 6,0	0,0 0,0	0,0 0,0
7 days..........	5,0 6,0	0,0 0,0	— —	0,0 5,6	0,0 0,0	— 0,0	4,4 5,5	5,0 5,6	0,0 6,0	0,0 0,0	0,0 0,0
8 days..........	0,0 0,0	0,0 0,0	— —	0,0 0,0	0,0 0,0	0,0 —	6,0 5,6	0,0 0,0	0,0 0,0	0,0 0,0	0,0 0,0

Up to the fifth day of incubation the mortality rate of the embryos did not exceed 4 per cent. This may be considered normal when it is remembered that the temperature of incubation was 37·5°, that the eggs were never cooled off or aired, and that owing to the presence of the artificial air space it was not possible to turn them. From the fifth day onwards the mortality rate increased steadily and this explains the omission of the virus titrations of embryos older than 16-17 days. Actually, however, no fewer than 7 embryos developed to maturity and hatched, the chicks in every case being deformed to a greater or lesser extent but all lived for more than 48 hours. No virus could be demonstrated in the brain of any chick 48 hours after hatching.

An attempt was made to compare the hatchability of eggs inoculated with horsesickness virus and controls seeded with sterile saline all being maintained under the same conditions. It was soon apparent that the mortality rate in both sets from the seventeenth day onwards was so high that significant figures could be obtained only after a statistical survey of numbers of fertile eggs greater than were available.

No histological examination of any organs was undertaken.

Conclusions.

From the experiments which have been described it may be concluded that the developing chick embryo constitutes a medium entirely suited to the propagation of neurotropic horsesickness virus. In the embryo the virus retains its essential neurotropism, multiplication occurring chiefly, if not exclusively, in the brain. The degree of multiplication in the chorio-allantoic membrane and in the body of the embryo, if it occurs at all, is negligible. The titre of the virus in the brain reaches a peak on the fourth or fifth day of incubation, after which there is a steady decrease so that by the eighth day only residual traces are detectable. Virus has not been found to multiply or persist in the chick after hatching.

These conclusions apply to only a single strain of virus (that known as 20449 which was used in its 181st passage in mice). It is not anticipated that the site of multiplication will differ in the case of other virus strains but it is possible that there will be some variation in the rate of multiplication and the maximum titre.

The virus appears to have no special lethal effect upon the embryo and after maximum development " dies " at a rate considerably faster than it does in a serum saline emulsion preserved at the same temperature (37·5° C.).

Discussion.

Before entering into a discussion of the interesting results obtained in this series of experiments it is necessary to state that three attempts were made to propagate a field pantropic strain of virus in a similar manner. Detailed protocols are omitted because of the entirely negative results obtained. Virus in the form of infective defibrinated blood haemolyzed by freezing and thawing, and clarified by centrifugation at 3,000 r.p.m. was seeded on to 9-day embryos in the usual manner. After incubation for four or five days, 3 embryos were harvested, divided into membrane, body and head, and a pooled 5 per cent. emulsion of each portion seeded separately on to other embryos. After three passages emulsions were injected separately into susceptible horses. No reactions were produced. This finding cannot be taken to imply definitely that pantropic virus will not multiply in the chick embryo, since the number of trials is too small, but it does indicate that a strain will be adapted only with difficulty.

It now becomes necessary to direct attention to the phenomenon of a virus, the original effect of which has been modified by intracerebral passage through mice, retaining its acquired neurotropism on propagation in the relatively poorly differentiated brain cells of the chick embryo, the mature chicken in turn being quite insusceptible to infection. Further, the multiplication of the virus proceeds rapidly to a point which apparently represents the saturation point of the host cells and then disintegration and death of the virus commences without having had any specific lethal effect. In other words, the findings represent an experimental symbiosis between a virus and a particular type of cell.

The fact that the virus has no lethal effect upon the embryo, and produces no easily detectable lesion on the membrane or in any of the organs, makes the use of the chick embryo quite unsuitable for the study of problems connected with immunity by the orthodox methods (Burnet, Keogh and Lush 1937). On the other hand, the chick embryo remains suitable for virus propagation in large quantities.

On numerous occasions 5 per cent. saline emulsions prepared from the brains of mice moribund after intracerebral infection of neurotropic virus strain 20,449 have been titrated and it is quite remarkable with what consistency ·05 c.c. of a one-ten-thousandth dilution constitutes 1 M.L.D. The weight of the brain of a mouse of the age used is approximately 0·45 grams which means that 100 c.c. of 5 per cent. emulsion can be obtained from about 12 mice. The " brain " of a chick embryo incubated from 12-14 days under the conditions described was found to weigh from 1·2-1·9 grams which means that 100 c.c. of virus emulsion of the same titre could be obtained from 3 or 4 embryos. This brings the propagation of attenuated virus for vaccine production in the embryo within practical bounds although it must be pointed out the work involved in seeding and harvesting 4 eggs is considerably greater than injecting and subsequently removing the brains from 12 mice. On the other hand, it is probable that provision could be made for sufficient fertile eggs to be available throughout the year whereas the incidence of a disease such as ectromelia, ring worm or mouse typhoid can wipe out an entire mouse stock. Were it not for the fact that the present routine horsesickness vaccine contains 5 strains of virus thus necessitating the use of at least 20 embryos for each batch of vaccine a change from mice to eggs would be indicated. In the present circumstances the egg method should be kept in reserve to replace the use of mice in case of dire necessity.

In the meantime there remains a considerable amount of essential work to be done e.g. it is necessary to determine whether all virus strains will grow in embryos as easily as strain 20449, what is the optimum period of incubation and the virus titre of all other strains, whether the degree of attenuation of the neurotropic virus remains unaltered for horses, and whether the antigenic response in horses remains the same. This work is proceeding and will be reported on in due course.

SUMMARY.

1. The technique for seeding eggs for the propagation of neurotropic horsesickness virus in the developing chick embryo is described in detail.

2. It is found that the virus retains its neurotropic character and multiplies in the brain of the embryo; little if any multiplication takes place on the chorio-allantoic membrane or in the body of the embryo.

3. The titre of the virus reaches a peak after 4-5 days incubation at 37° C. after which there is a progressive fall in virus content; after 8 days only traces of virus are detectable.

4. The virus produces no macroscopically detectable lesion on the membrane and only slight heamorrhagic infiltration in the brain.

5. The virus exerts no specific lethal action on the embryo. Several chicks have hatched and were found to retain no virus in the brain.

6. The embryo mortality ratio amongst seeded eggs during the first 5 days of incubation does not exceed 4 per cent.

7. The possibility of using the chick embryo method for the propagation of virus for vaccine production is discussed.

ADDENDUM.

The continuation of this work on the propagation of neurotropic horsesickness virus was discontinued for approximately a year. An attempt was then made by my colleague, Dr. J. H. Mason, to cultivate two other neurotropic attenuated strains in chick embryos but without success. In the light of the above report which actually constituted the third separate adaptation to chick embryos these failures appeared so remarkable that parallel control experiments were run by me using strain 449 in its 246th mouse passage. The virus was lost by the third egg passage. The work was then repeated using the original strain 449 generation 181 that had been stored in a refrigerator at 4° C. and was still fully virulent for mice. Again no multiplication could be demonstrated and the virus died out by the fourth egg passage. Eggs from the same source but naturally from different birds were used and it is believed that the identical technique was followed but the reason for the consistent failure remains obscure.

Bearing in mind the ease with which the virus had been cultivated for 17 passages these quite unexpected findings are the present subject of close investigation. It would appear that adaptation to the chick embryo is not the simple matter that the above work had lead us to believe.

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Section II.

Parasitology.

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South African Helminths.—Part III.

Some Mammalian and Avian Cestodes.

By R. J. ORTLEPP, Section of Parasitology, Onderstepoort.

Fam. DAVAINEIDAE Fuhrmann, 1907.

Sub-fam. DAVAINEINAE Braun, 1900.

Raillietina (Skrjabinia) deweti sp. nov.

The available examples of this species consisted of one entire specimen and the scolex and fragments of another specimen; these were collected by Mr. de Wet, veterinary student at this institute, and placed at the writer's disposal for determination.

The entire specimen is 48 mm. long with a maximum breadth of 0·98 mm. It is built up of 31 segments plus a scolex and short neck; all the segments, except the few anteriormost, are much longer than broad, and the posteriormost segments have a superficial resemblance to the ripe segments of *Joyeuxia fuhrmanni* from carnivores.

The scolex has a diameter of 0·174 mm. and carries four oval suckers, 0·15 mm. long by 0·13 mm. across; the suckers carry no hooklets, but whether this is normal or whether they have been lost, it is not possible to say. The rostellum carries a double row of about 200 typical hammer-shaped hooks, each having a length of 0·014 mm. The neck is about 0·4 mm. long and in the complete specimen the first segment is broader than long, measuring 0·19 mm. by 0·07 mm.; in the incomplete specimen the first segment is longer than broad, measuring 0·2 mm. long by 0·15 mm. broad. In the complete specimen segments 19 to 22 are mature and segments 29, 30 and 31 are gravid. The largest mature segment is 2·1 mm. long by 0·58 mm. broad and the posteriormost gravid segment is 4·4 mm. long by 0·62 mm. broad.

The ventral excretory canals are well developed and may attain a diameter of 0·05 to 0·07 mm.

The genital atria alternate irregularly and are situated just anterior of the middle of the segment, their position roughly dividing the segment into the ratio of 4:5. The pore leads into a small genital atrium at the base of which open the male and female ducts. The cirrus sac is large and muscular and extends obliquely inwards and forwards, crossing the excretory canals, and almost reaches the midline of the segment (Fig. 1 and 2); it has a fusiform shape and in mature segments reaches a length of 0·32 mm. by 0·052 broad; a few weak retractor muscles are attached to its base. The cirrus is well developed, about 0·2 mm. long and 0·015 mm. thick and is densely covered with minute spines; it leads into a somewhat thick-walled and convoluted vesicula seminalis interna which on emerging from the cirrus sac passes backwards and becomes much coiled; the coils are surrounded by darkly staining cells which possibly are prostatic in nature. The testes, of which there are 80 to 100, are all

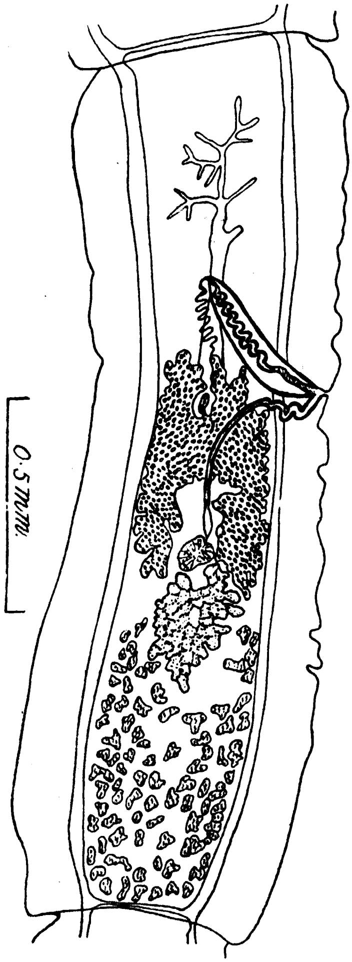

Fig. 1.—*Raillietina* (*Skrj.*) *deweti* sp. nov. Mature segment.

situated in the posterior third of the segment behind the ovary; the anteriormost testes, however, extend lateral of the yolk gland; they are irregularly shaped, but whether this is their normal shape or due to contraction is not certain.

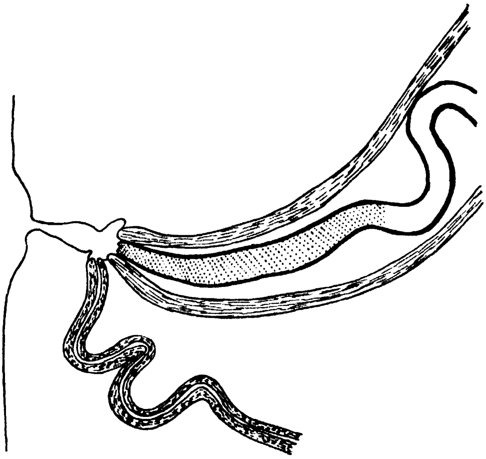

Fig. 2.—*Raillietina* (*Skrj.*) *deweti* sp. nov. Terminal portions of cirrus pouch and vagina.

The female genital glands occupy the middle third of the segment. The ovary is in the shape of an inverted U and consists of many lobules; it occupies the whole space between the excretory canals; it is about 0·5 mm. long by 0·3 mm. broad; between the tips of the U there is a small somewhat globular shell gland having a diameter of about 0·07 mm. The yolk gland is large, somewhat heart shaped and is situated immediately behind the ovary; it is almost 0·3 mm. long with a maximum breadth of 0·2 mm. The vagina is thick walled and opens into the genital atrium on the same level but behind the opening of the cirrus pouch; it is thrown into several loops until it passes over the excretory canal, after which it passes in a curve posteriorwards between the two ovarian limbs; its posterior end is slightly dilated to form a spindle-shaped reseptaculum seminis after which it joins the shell gland. The uterus makes its appearance in the mature segments as a median canal extending forwards from the level of the ovary; it gives off numerous irregular side branches; when the genital glands begin to disappear it also extends posteriorwards also giving off numerous lateral branches; these eventually fill the whole proglottid between the excretory canals, and in it the numerous small eggs are lodged. In the ripe segments the uterus disappears and the eggs are seen to lie singly filling the whole inter-excretory canals area and not aggregated into clumps to form egg capsules. The eggs are small and rounded and only measure 0·014 to 0·015 mm. in diameter; in no case were any embryonic hooks made out.

Affinities.—The hammer-shaped rostellar hooks, irregularly alternating genital pores and the absence of egg-capsules containing several eggs places this species in the subgenus *Skrjabinia* Fuhrmann, 1920. According to Fuhrmann (1932) seven species of this subgenus have been described from galliform birds, and, except for *R.* (*S.*) *bolivari* (Lopez-Neyra, 1929) the writer has been able to obtain some data concerning their morphology. The species *R.* (*S.*) *cesticillus* (Molin, 1858), *R.* (*S.*) *maroteli* (Neveu-Lemaire, 1912) and *R.* (*S.*) *ransomi* (Williams, 1931) differ from the writer's species in their greater number of rostellar hooks (ca. 500) and in their much smaller cirrus pouches and smaller number of testes. *R.* (*S.*) *circumvallata* (Krabbe, 1869) and *R.* (*S.*) *polyuterina* (Fuhrmann, 1908) have ca. 200 hooks, 0·016 mm. long, but can be separated from the writer's species by their smaller number of testes (20 and 40 respectively) and much smaller cirrus pouches; the small cirrus pouch also distinguishes *R.* (*S.*) *retusa* (Clerc, 1903) from the above described species. The elongated nature of the segments, the disposition of the genital glands and the large size of the cirrus pouch seem to ally the writer's species with *R.* (*S.*) *bovini* (Megnin, 1899) from columbiform birds, but these two species can be easily separated in that the latter species has only 120 rostellar hooks, the ovaries are smaller and not U-shaped and the testes are only about 30 in number.

Specific diagnosis.—Davanineinae provided with ca. 200 rostellar hooks, 0·014 mm. long; genital pores irregularly alternating; majority of segments much longer than broad; about 100 testes in posterior third of segment behind ovary; cirrus sac large, almost reaching mid-line of segment; ovary an inverted U, large and much lobulated; yolk gland behind ovary, large and somewhat heart-shaped; vagina thick-walled and distal portion convoluted; uterus arises as a median stem with numerous side branches. Eggs single and not forming egg capsules containing several eggs.

Host: *Numida* sp.

Location: Small Intestine.

Locality: Lady Grey, Cape Province.

Type in the Onderstepoort Helminthological collection.

Sub-fam. IDIOGENINAE Fuhrmann 1907.

*Schistometra korhaani** sp .nov.

This species is represented by two mature specimens collected from a white-quilled Korhaan, Orange Free State, and two immature specimens from a Grey Korhaan, Transvaal. Only one of the mature specimens contained a head which, for some reason unknown, had been twisted ventralwards and flattened *en face;* however, the hooks were still present.

The specimens are a dirty brownish grey and do not show a yellowish colour in their end segments as described for other members of this genus. The largest specimen is 70 mm. long, and has a maximum breadth of 2·6 mm. behind its middle, and a thickness of 1·6 mm.; the other mature specimen was sectioned, transversely and horizontally; it was 63 mm. long, and had a maximum breadth of 2·8 mm. and a thickness of 1·9 mm. the flattened scolex is 0·85 mm. by 0·75 mm. across; the two scolices from the immature specimens are quadrangular and seen from the front have a laterolateral width of 0·4 and 0·46 mm. and a dorso-ventral width of 0·37 and 0·4 mm. respectively. The rostellum was in all cases retracted; it is flattened dorso-ventrally and in the unflattened heads it was 0·28 mm. broad by 0·15 mm. high; it is armed by numerous small hammer-shaped hooks arranged in two rows (Fig. 3); these rows are arranged in an irregular zig-zag and do not meet laterally, i.e. the dorsal and ventral rows of hooks are separated from each other by a break; two heads, which apparently still retained all their hooks, carried respectively just over 700 and 800 hooks each. These hooks are 0·010 mm. long. The four suckers occupy the four corners of the head; each is provided with two lateral appendages as described for the genus *Octopetalum;* they are unarmed and in the unpressed heads have a diameter of 0·17 mm. and a depth of 0·12 mm.

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* "Korhaan" is the Afrikaans name for a Bustard.

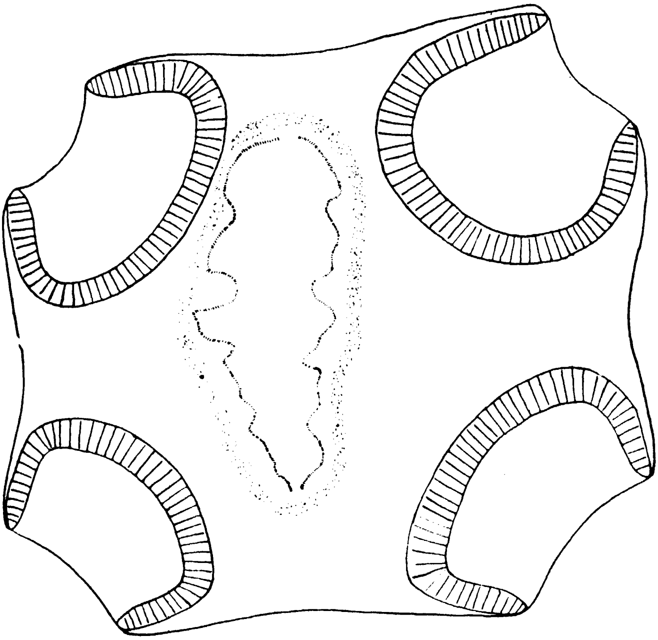


Fig. 3.—*Schistometra korhaani* sp. nov. Front view of scolex showing arrangement of hooks.

Round the base of the rostellum there are numerous small hooks, having the shape of a fish-hook. They are 0·003 mm. long with a hook about 0·001 mm. long.

In transverse section it is noted that the longitudinal musculature is very strongly developed (Fig. 4); it forms a layer about 0·3 mm. thick surrounding the parenchyma, this layer being built up of numerous columns of muscle bundles, each column composed of 5 to 7 bundles; the outermost bundles are the smallest, being composed of 1 to 3 fibres each, but passing inwards the bundles become larger and the innermost bundles may each be built up of as many as 80 fibres, each 0·003 to 0·008 mm. in diameter. On the outer and inner margin of this muscle layer there is a thin layer of circular muscles, and the outer surface is bounded by a cuticle reaching 0·013 mm. in thickness. The dorso-ventral musculature is very poorly developed and consists of only a few isolated fibres.

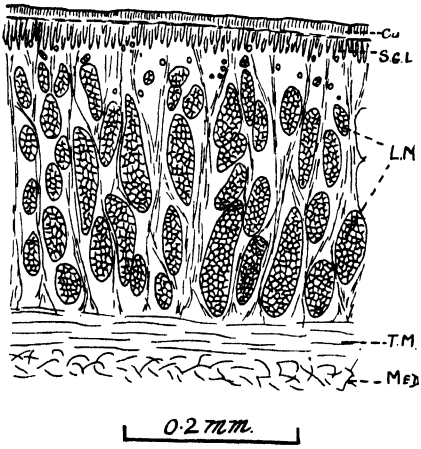


Fig. 4.—*Schistometra korhaani* sp. nov. Transverse section of cortex (Cu = cuticle; S.G.L. = subcuticular glandular cells; L.M. = longtitudinal muscle bundles; T.M. = transverse muscles; Med. = medulla).

The ventral excretory vessel is large and thin-walled and runs more or less straight; in mature segments it has a diameter of 0·13 to 0·17 mm.; and at the posterior end of each segment a valve projects into its lumen as figured for *Schistometra conoides;* the dorsal excretory vessel is much smaller, having a diameter of at most 0·018 mm.; it runs a somewhat zig-zag course, has a thicker wall and is surrounded by darkly staining cells. The nerve cord is quite distinct and lies just lateral of the ventral excretory canal and has a thickness of 0·025 to 0·03 mm.

Except for the posterior segments, the segments are all very much broader than long; the posterior edge of each segment is extended backwards to form a collar covering about half of the segment behind. The genital atria are practically unilateral but a few contiguous atria are present on the opposite side; they are situated in the anterior half of the segment; the genital ducts have a peculiar course in that they pass between the excretory canals and dorsal of the nerve cord or dorsal of the excretory canals and nerve.

The cirrus pouch is muscular and oblong and does not reach the excretory canals; it is from 0·2 to 0·3 mm. long by 0·04 to 0·045 mm. thick; the cirrus is delicate and appears to carry minute spines. The vas deferens is coiled inside the cirrus pouch and after crossing the excretory canals it becomes densely coiled and is surrounded by deeply staining prostatic cells. There appear to be from 40 to 60 testes, 0·035 by 0·023 mm. to 0·042 by 0·015 mm. size, and confined to the posterior half of the segment; they occupy a vertical plane, two to three testes deep, behind the ovary, and the plane may be one to three testes broad; they do not extend beyond the excretory canals, and in a horizontal section 12 to 20 testes may be seen in a single section of a segment.

The vaginal aperture is situated on the same level but behind the opening of the cirrus pouch; both open into a deep genital sinus about 0·1 mm. deep. The ovary is slightly poral in position and consists of two lobes; the shell gland lies between these two lobes but dorsal, and the yolk gland lies posterior of the aporal ovarian lobe. The uterus makes its first appearance as a transverse structure in front of the testes; its growth is more rapid laterally, less so dorso-ventrally and least anteriorly; it extends laterally to the excretory canals and anteriorly to about the middle of the segment; irregular antero-posterior septa portion off its lumen into a number of compartments.

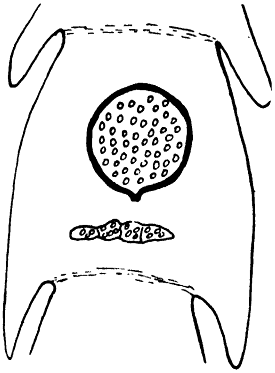


Fig. 5.—*Schistometra korhaani* sp. nov. Horizontal section of ripe segment showing paruterine organ.

The paruterine organ arises as a streak of deeply staining cells along the anterior margin of the segment; it grows posteriorly but its centre grows quicker than its sides. This thickened central plug grows and presses on the anterior face of the uterus and eventually by further growth forces the anterior wall of the uterus back on to its posterior wall; in this way in older segments there are apparently two separate uteri, one on either side of the paruterine organ, but careful examination will show that these two sacs are joined to each other by the adjacent anterior and posterior uterine walls. With the continued growth of the paruterine organ the segments become longer and may eventually become 1·4 mm. long by 1·1 mm. broad and 1·2 mm. thick; in these segments the adjacent uterine walls, behind the paruterine organ, again become separated and the organ itself becomes globular and hollow (Fig. 5); just before the segments become liberated an opening is formed on the posterior face of the paruterine organ and through a rupture in the uterus the eggs pass singly from the uterus into the organ. The eggs almost fill the organ, the rest of the organ being filled by a structureless cement-like material. In fully mature segments the paruterine organ reaches a length of

0·52 mm. by 0·435 mm. across. The eggs are provided with three coats, the 2nd forming the egg shell; they are rounded 0·037 to 0·04 mm. in diameter with an embryo measuring 0·032 mm.

Affinities.—The type of rostellar hooks and the presence of a paruterine organ places this species in the sub-family Idiogeninae Fuhrmann, 1907. The well developed longitudinal muscles, the disposition of the testes in a vertical plane behind the female glands, the presence of appendages on the suckers, and the presence of values in the excretory system places it in the genus *Schistometra* Cholodkovsky, 1912. Three species of cestodes have been assigned to this genus, namely *S. conoideis* (Bloch, 1702), *S. macqueeni* (Woodland, 1930) and *S. wettsteini* Weithofer, 1916, all of which occur in otidiform birds. A description of the last named species is unfortunately not available.

From Skrjabin's (1914) redescription of *S. togata* Cholod., 1912 [=*S. conoideis* (Bloch)] it is apparent that this species differs from the wrier's material in the number and arrangement of the rostellar hooks and in the irregularly alternating arrangement of the genital pores. In *S. macqueeni* the rostellar hooks are arranged in a single row, the genital pores irregularly alternate and the uterus breaks up into capsules containing several eggs.

Specific diagnosis.—Idiogeninae with thickened strobila, and well developed longitudinal muscles and segments, except the last few, much broader than long. Scolex with four unarmed suckers each carrying a pair of appendages, and rostellum with numerous (700-800) hooks arranged in a double zig-zag row not meeting laterally; numerous minute hooklets covering base of rostellum. Genital pores mostly unilateral. Genital ducts pass between or dorsal of excretory canals; 40-60 testes in a vertical plane in posterior half of segment; ovary two lobed, slightly poral in position and anterior to testes; uterus transverse and divided by septa into a number of compartments. Paruterine organ globular and receives eggs prior to being detached.

Host: *Afrotis afroides afroides* (A. SMITH) and *Eupodotis barrowi* (GRAY).

Location: Small intestine.

Locality: Orange Free State and Transvaal.

Types, in the Onderstepoort Helminthological collection.

Fam. ANOPLOCEPHALIDAE Fuhrmann, 1907.

Sub-fam. LINSTOWINAE Fuhrmann, 1907.

Inermicapsifer leporis sp. nov.

About 50 specimens of this small cestode were recovered from the small intestine of a vlakhaas—*Lepus capensis vernayi* Roberts, shot at Fauresmith, O.F.S. The specimens were well extended and were studied both in section and as *toto* mounts.

The length varies from 15 to 25 mm. with a maximum breadth towards the posterior end of 1·01 to 1·07 mm.; the scolex is fairly large and squarish and has a cross diameter of 0·59 to 0·64 mm.; the suckers are rounded, prominent and have a diameter of 0·23 to 0·28 mm. The head is followed by a short neck 0·23 to 0·3 mm. long by 0·34 to 0·41 mm. wide. The segments, of which there are 75 to 90, are at first very much wider than long; as they reach maturity, however, the length increases so that in mature segments they are only slightly more than twice as broad as long; (0·73 by 0·3 mm.) further back the relations change so that when ripe the segments are actually longer than broad (0·73 to 0·82 mm. broad by 0·84 to 0·9 mm. long).

The genital rudiments make their appearance very soon after the demarcation of the youngest segments; the first to appear are the rudiments of the female glands and the vagina and cirrus; about 15 segments further back the testes appear and from about the 35th to 40th segment the segments became mature. Only the last half dozen segments can be considered as ripe. The genital pores are all unilateral and situated at the centre of the lateral margin; in ripe segments, however, they may occupy a slightly more posterior position. The genital ducts all pass dorsal of the nerve and ventral excretory canal.

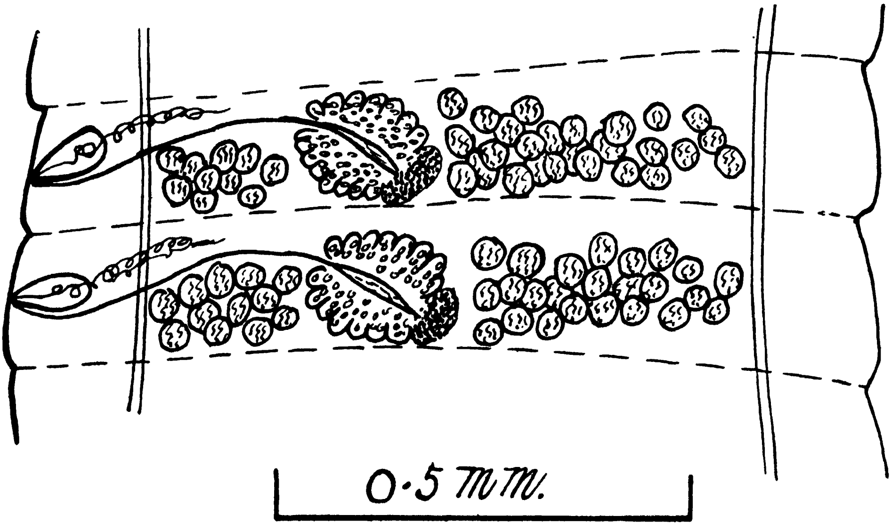


Fig. 6.—*Inermicapsifer leporis* sp. nov. Mature segment.

The testes (Fig. 6) of which there are from 35 to 40 in each segment, are, when mature, hollow oval bags having their germinal cells aggregated towards one side and the rest of the bag filled with sperms; they measure from 0·05 to 0·065 mm. by 0·04 by 0·48 mm. When they make their first appearance they are arranged in two groups separated by the female gland rudiments; as they reach maturity they enlarge and may pass posterior of the ovarian rudiments thus forming a continuous layer lateral and posterior of the ovary. However, this state only lasts through a few segments because, as the ovary matures and enlarges, it pushes the testes apart so that there is again a poral and an aporal group. The poral group consists of from 10 to 13 testes, the remainder being aporal; they reach but do not pass over the excretory canals, neither are they found anterior of the female glands; they form a layer one to two testes deep. The cirrus sac is muscular and oval 0·11 to 0·15 mm. long with a maximum diameter in its inner third of 0·05 mm.; it may reach but does not cross the excretory canals. The cirrus is very weak and unarmed; a small vesicular seminalis interna is present. After crossing the excretory canal the vas deferens widens, is filled with sperms and is thrown into several dorso-ventral loops.

The vagina opens to the exterior posterior and ventral of the cirrus sac; it is muscular, has its lumen lined by delicate cilia, and extends to the excretory canal. Beyond the excretory canal it loses its muscular nature and enlarges to form a thin-walled and slightly convoluted receptaculum seminis. The ovary is a multilobulate organ, slightly poral in position, and has its lobes limited to its lateral and posterior margins; it is about 0·16 mm. long by 0·17 mm. broad. The yolk gland is somewhat oval, 0·09 mm. broad by 0·07 mm. long and is situated posterior and ventral of the ovary, slightly towards its aporal margin. The uterus arises as a transverse tube, but it very soon breaks up to form 40 to 50 egg capsules each containing 3 to 10 eggs; these eventually occupy the whole of the segment and even extend lateral of the excretory canals.

The cuticle is 0·004 to 0·006 mm. thick; internal to it and extending through the whole thickness of the cortical parenchyma there are numerous irregularly scattered longitudinal muscle fibres, not arranged in any definite layer. Vertical and transverse muscles are also well represented by irregularly scattered figres.

The excretory system is represented by the usual two longitudinal canals, which, however, are situated on the same level; the ventral excretory canal is from 0·21 to 0·29 mm. from the edge of the segment in mature and ripe segments, and has a diameter of about 0·02 mm.; the dorsal excretory canal is found about midway between the ventral canal and the edge of the segment; it is very small and has a diameter of only about 0·006 mm.

Affinities.—The above species forms a transition between the group *settii* and the group *hyracis* in that the two groups of testes are not so widely separated as in the former group, and also because prior to the maturation of the ovary the testes meet posterior of the ovary as is seen in the latter group. However, this latter condition is only transitory, and consequently the above described species can be referred to the *settii* group.

Of the twelve species of this genus which have been rescribed from mammals, there is only one, *I. settii* Janicki, 1910 from *Procavia capensis*, in which the testes are arranged in two groups.

The writer's specimens differ from Janicki's species in that the two groups of testes are not so distinctly separated, the number of testes is smaller, there are more egg capsules per segment and the genital pores are situated in the centre of the lateral margin.

Specific diagnosis.—Linstowinae up to 25 mm. long characterised by having 36 to 40 testes arranged in a poral group of 10 to 13 testes and the remainder situated aporally; no testes anterior of the ovary, and prior to maturity of ovary a few testes may be posterior of the ovary. Ovary slightly poral in position; receptaculum seminis present; 40 to 50 egg capsules to each segment, these extending laterally over the excretory canals.

Host: Lepsus capensis vernayi Roberts.

Situation: Small intestine.

Locality: Fauresmith, Orange Free State.

Types in the Helminthological Collection, Onderstepoort.

Fam. DILEPIDIDAE Fuhrmann, 1907.

Sub-fam. DILEPIDINAE Fuhrmann, 1907.

Catenotaenia geosciuri sp. nov.

This species is represented by one worm 93 mm. long and the scolex and fragments of what probably represents a second specimen. Entire specimens are probably considerably longer than the longest worm present, because in this worm the hindmost segment was only about half the length of detached segments, and neither did it contain any eggs. The largest worm was mounted *in toto* and from it, except where otherwise stated, the following details were observed. The worm consists of 37 segments which progressively increase in length posteriorly. The anteriormost segment is slightly broader than long (0·278 mm. by 0·19 mm.) and so is the second (0·282 mm. by 0·232 mm.); the third is longer than broad (4·27 mm. by 1·24 mm.); the last segment is the largest measuring 7·3 mm. long by 1·45 mm. broad in its middle; detached segments were much longer, the largest measuring nearly 15 mm. long by 1·6 mm. broad. These elongated segments give the strobila a superficial appearance very similar to that seen in members of the genus *Dipylidium*.

The head is relatively small and carries four somewhat rounded and small suckers; in the mounted specimen the head measured 0·27 mm. across and the suckers had a diameter of 0·087 mm.; the unmounted head measured 0·348 mm. across and the suckers had a diameter of 0·128 mm. In both scolices a rostellum and hooks were absent. The neck is elongate and is slightly thinner than the scolex; it measured 1·3 mm. long and 0·236 mm. across in the mounted and 1·6 mm. long and 0·29 to 0·365 mm. across in the unmounted specimen.

The longitudinal muscles are fairly well developed and in cross section are seen to be arranged in a single layer surrounding the medulla; it consists mostly of isolated muscle fibres, but in some places there appears to be a tendency for 4 to 7 fibres to be aggregated together to form bundles. Circular and dorso-ventral muscles appear to be poorly developed.

The excretory system consists of two longitudinal canals passing down the length of the strobila; these do not form a complicated network as described for *C. lobata* Baer and *C. symmetrica* Baylis. The ventral canals are large and increase in diameter posteriorwards; in section they have a diameter of 0·03 to 0·04 mm. in mature segments and 0·072 to 0·09 mm. in ripe segments; these canals are joined to each other by a transverse canal at the posterior end of each segment. The dorsal canals are small, have a diameter of 0·009 to 0·014 mm. and are situated dorso-lateral of the ventral canals. The nerve cord lies just lateral of the ventral canals. The genital ducts pass dorsal of the ventral canal and nerve cord.

The genital pores alternate irregularly and are situated far forwards at about the junction of the 1st and 2nd fifths of the segment. The cirrus sac is somewhat pearshaped and is from 0·2 to 0·25 mm. long with a diameter of 0·11 to 0·13 mm. (Fig. 7); it crosses the excretory canals. The cirrus is weakly muscular, is unarmed, has its lumen lined by cuticle and is from 0·04 to 0·045 mm. thick. A vesicula seminalis interna is present. The testes are numerous and are all situated in the posterior half of the segment behind the female glands; there appear to be about 200 testes from 0·064 to 0·087 mm. in diameter.

A striking characteristic of this species is the excessive development and position of its vagina; it opens into the genital pore just posterior of the opening of the cirrus sac; it passes transversely inwards until it crosses the excretory canals after which it passes backwards, more or less parallel to the ventral canal and is thrown into a few loops during its course. It is thick walled and has a diameter of about 0·075 mm. to 0·085 mm.; its lumen has a cuticular lining; it is surrounded by darkly staining prostatic cells, and is about 1·3 to 1·5 mm. long. Its posterior extremity widens out to form a globular receptaculum seminis, situated just in front of the yolk glands; the receptaculum seminis is small and inconspicuous in mature segments but after the dissolution of the ovary and yolk glands it increases in size and may attain a diameter of 0·23 to 0·29 mm. The female glands are confined to the anterior half of the segment; the ovary is large and multilobed and occupies an aporal position; it has a maximum length of 1·46 mm. by 0·73 mm. broad, and extends slightly posterior of the yolk gland. The yolk gland is porally situated towards the posterior end of the ovary; it is multilobed somewhat rounded organ attaining a length of 0·427 mm. by 0·45 mm. broad. The uterus first makes its appearance in mature segments as a centrally placed hollow tube extending forwards anterior of the ovary; in older segments a similar tube passes backwards towards the posterior end, and when the sexual glands begin to disappear sacculations or lateral branches are formed on either side of this central tube; in ripe segments these lateral sacculations number about 40 on either side and the majority show secondary indentations (Fig. 8). Numerous eggs are present; the outer membranous envelope has diameter of 0·023 to 0·025 mm.; the inner cuticular shell is oval, 0·015 to 0·0175 mm. long by 0·11 mm. broad, and the embryo fills its lumen. The six hexacanth hooks are small and only measure 0·005 to 0·006 mm. in length.

Affinities.—The absence of an excretory network allies this species to *C. pusilla* (Goeze, 1782) and *C. dendritica* (Goeze, 1872) and distinguishes it from the two species *C. lobata* Baer, 1925 and *C. symmetrica* Baylis, 1927. It has about the same number of testes as in *C. dendritica* (approximately 200 to 250) but many more than in *C. pusilla* (approximately 70). The writer's species, however, may be differentiated from *C. dendritica* by the nature and position of its vagina, the more anterior position of the genital pores and by its greater number of lateral uterine branches. The writer has not been able to compare his species with *C. rhombomidis* Schulz and Landa, 1934, these authors' description not being available in South Africa.

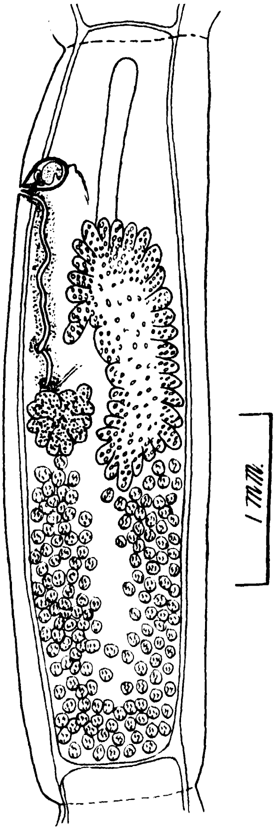


Fig. 7.

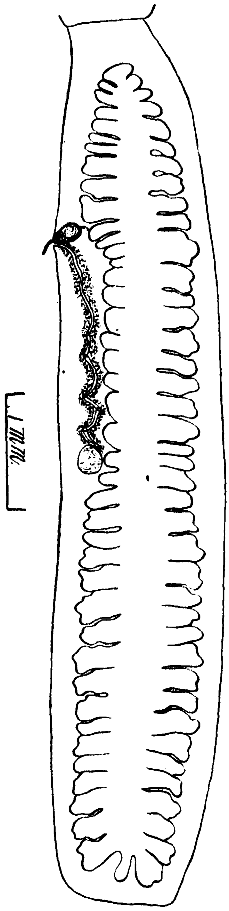


Fig. 8.

Fig. 7.—*Catenotaenia geosciuri* sp. nov. Mature segment.
Fig. 8.—*Catenotaenia geosciuri* sp. nov. Ripe segment showing uterus.

Specific diagnosis.—Dilepidinae without hooks or rostellum; strobila probably considerably longer than 93 mm.; segments, except for the first few, all longer than broad; posterior segments may be

15 mm. long; excetory system of small dorsal and large ventral canals, latter joined to each other by transverse canal at posterior end of segment; excretory network of canals absent. Genital pores alternate irregularly and are situated far forwards; cirrus sac pear-shaped, crosses excretory canal and contains an unarmed cirrus and vesicula seminalis interna; testes about 200 in number and situated behind female glands in posterior half of segment. vagina long, muscular, surrounded by darkly staining prostatic cells and passes backwards more or less parallel to lateral margin of segments; ovary large, multilobate and aporal; yolk gland somewhat rounded and lobate, situated porally at side of posterior extremity of ovary; reseptaculum seminis present; uterus a median stem with about 40 sacculations on either side; eggs numerous, outer envelope 0·023 to 0·025 mm. diameter and inner egg shell 0·015 to 0·0175 mm. diameter.

Host: Geosciuris capensis capensis (Kerr.)

Location: Small intestine.

Locality: Lady Grey, Cape Province.

Types in the Onderstepoort Helminthological Collection.

Onderstepoortia taeniaeformis gen. & sp. nov.

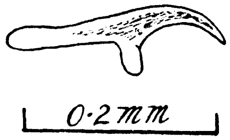
Four specimens of this species were obtained from a single stone curlew shot in the grounds of this institute; besides this species the intestine contained more than a hundred specimens of a cestode which the writer thinks is identical with *Paricterotaenia delachauxi* (Baer, 1925).

The maximum length is 41 mm.; the longest specimen has 65 segments and in it the genitalia make their appearance in the 40th segment; the 53rd to 60th segment are mature and the last segment has lost all its genitalia but no uterus or eggs are present. A specimen 38 mm. long has 76 segments and the genitalia appear in the 45th segment; segments 59 to 67 are mature and the last segment contains eggs. The maximum breadth is 2 mm. and the segments are all broader than long except the posteriormost where they may reach a length of about 3 mm. by 1·9 mm. broad.

The scolex is large and reaches 1·1 mm. across; the four suckers are rounded, have a diameter of about 0·28 mm. and are tilted slightly forwards. The rostellum is massive up to 0·34 mm. diameter and carries a single circlet of 30 to 32 taenoid hooks 0·22 to 0·228 mm. long from the tip of the blade to the tip of the handle (Fig. 9); the blade and the handle are more or less in a straight line and the guard forms almost a right angle with this line. The hooks vary slightly in size, alternate hooks having slightly shorter and narrower handles than those adjacent.

The neck is short and is only 0·3 to 0·39 mm. long with a maximum breadth of 0·6 mm.; mature segments are from 1·1 to 1·5 mm. long by 1·8 to 2 mm. broad; the posteriormost segment may reach a length of 3mm. by 1·9 mm. broad.

In transverse sections of mature segments the following anatomical details are observed (Fig. 10); the cuticle has a thickness of 0·007 mm. and this is followed by a very thin layer of transverse muscle fibres; the subcuticular glandular cells form a layer about 0·04 mm. thick and consists of large club-shaped cells; the longitudinal muscles in the cortex form a layer about 0·14 mm. thick and consists of large bundles of muscle fibres, the bundles being generally 3 deep; the outer bundles are smaller and consist of 15 to 20 fibres each whereas the inner bundles are larger and consist of 40 to 50 fibres each; each fibre may attain a diameter up to 0·009 mm.; the parenchyma between this layer and the glandular cells is from 0·042 to 0·049 mm. thick and contains very few isolated fibres; more isolated fibres are however present in this region towards the ends of the segment. The inner transverse muscles form a layer only 0·012 to 0·014 mm. thick. The central and dorsal excretory vessels are relatively large, the formar having a diameter of 0·026 to 0·032 mm. and the latter of 0·01 to 0·012 mm.; the formar is situated 0·29 to 0·38 mm. from the edge of the segment. The nerve cords lie just outside the ventral excretory canals, are more or less rounded and have a diameter of 0·058 to 0·073 mm. The genital ducts pass between the excretory canals and dorsal of the nerve.



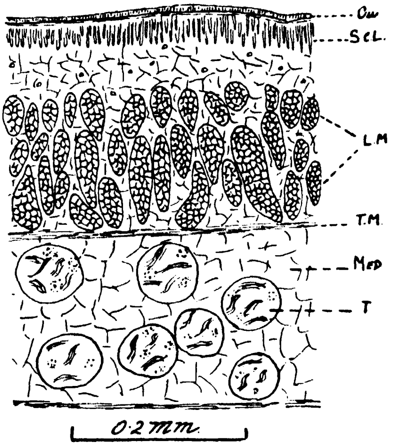


Fig. 9.—*Onderstepoortia taeniaeformis* gen. & sp. nv. Rostellar hook.

Fig. 10.—*Onderstepoortia taeniaeformis* gen. & sp. nov. Transverse section of medulla and cortex. (T = testis; other lettering as in Fig. 5.)

The genital atria are small and inconspicuous, alternate irregularly and are situated in the anterior half of the segment at about the junction of the first and second thirds. The cirrus sac is muscular and club shaped and crosses the excretory canals (Fig. 11); it is from 0·278 to 0·29 mm. long with a maximum thickness of 0·052 mm.; the vas deferens forms only a few coils inside the cirrus sac but after emerging it forms a dense mass of coils. The testes fill up the whole segment not occupied by the female glands and male and female genital ducts; they number from 120 to 150 and are limited laterally by the excretory canals, and occur throughout the thickness of the medulla forming a layer two to three testes deep; they are rounded and large and have a diameter of 0·067 to 0·073 mm. A vesicula seminalis interna and externa are absent.

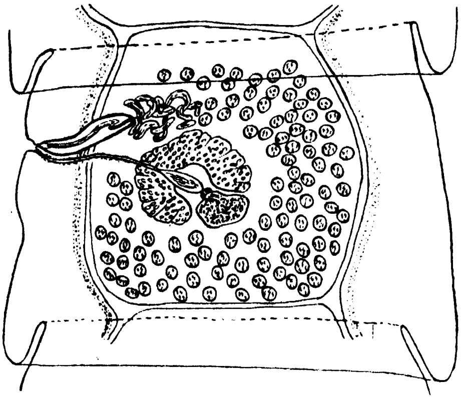


Fig. 11.—*Onderstepoortia taeniaeformis* gen. & sp. nov. Mature segment.

The vagina opens into the genital atrium just behind the cirrus sac; it is muscular and is surrounded by darkly staining cells; its inner end is expanded to form a large and oval receptaculum seminis which may reach a length of 0·232 mm. by 0·104 mm. across. The ovary is a crescentic multilobed organ, slightly poral in position; the yolk gland is large and lies posterior-lateral of the ovary forming a kind of plug to this organ. The shell gland is very small and is partially embedded in the dorsal portion of the yolk gland. The uterus appears to be very transitory because no signs of it were seen prior to the appearance of the eggs; in sections of egg containing segments the uterus has broken up into a number of small chambers, each of which contains a single egg. The eggs fill up the whole segment between the excretory canals; they are rounded and thin shelled and have a diameter of about 0·058 mm.; the onchosphere is up to 0·045 mm. large and its hooks are 0·026 mm. long.

Affinities.—The presence of an armed rostellum with taenoid hooks and a uterus which breaks up to form egg capsules places this species in the sub-family Dipylidiinae (Stiles, 1896) of the family Dilipidae Fuhrmann, 1907. Among the known genera of this subfamily the writer's material appears to have its nearest relatives among the members of the genus *Choanotaenia* Railliet, 1896; this is supported by the presence of a single crown of rostellar hooks, irregularly alternating genital atria, genital ducts passing between the excretory canals and dorsal of the nerve and the uterine capsules containing a single egg. Also the arrangement of the testes is very similar to that seen in *Choanototaenia cingulifera* (Krabbe, 1869) except that in this species testes are not present anterior of the coils of the vas deferens. The taenoid shape of the hooks would tend to ally the writer's species to those of the genus *Paricterotaenia*

Fuhrmann, 1932 (Dilepidinae Fuhrmann, 1907) because in *P. macracantha* Fuhrmann, 1908 hooks of the same shape are present and these are also arranged in a single circle, but the presence of uterine egg capsules and testes anterior of the female glands excludes the writer's species from this genus.

The shape of the rostellar hooks and the arrangement of the testes encircling the female glands and passing anterior of the coils of the vas deferens are characters which, as far as the writer could ascertain, have not been recorded for any known member of the genus *Choanotaenia* and the writer feels that these characters are of sufficient weight to merit the creation of a new genus—*Onderstepoortia*—for the reception of this species. This genus would be characterised as follows: *Dipylidiinae of medium size having a single crown of taenoid hooks; genital atria irregularly alternating; genital ducts pass between excretory canals and dorsal of nerve; testes encircle female glands and pass anterior of coils of vas deferens; uterus forms capsules each containing a single egg.* Type: *O. taeniaeformis* sp. n. from *Burhinops capensis capensis*, Onderstepoort, Transvaal.

Specific diagnosis.—Dipylidiinae reaching a length of 41 mm. having a rostellum carrying a single crown of 30 to 32 taenoid hooks 0·22 to 0·228 mm. long; cirrus sac crosses the excretory canal; testes 120-150 in number, rounded, practically fill the whole segment, ensircle the female glands and pass in front of the coils of the vas deferens; ovary slightly poral and crescentic; receptaculum seminis large; uterus breaks up to form egg capsules each containing a single egg; eggs thin shelled, 0·058 mm. in diameter.

Host: Burhinops capensis capensis, (Lcht.).

Location: Small intestine.

Locality: Onderstepoort, Transvaal.

Types in the Onderstepoort Helminthological Collection.

Fam: HYMENOLEPIDIDAE Fuhrmann, 1907

Sub-fam.: HYMENOLEPIDINAE Perrier, 1897.

Hymenolepis suricattae sp. nov.

This species is represented by several fragments originating from at least five worms as represented by their heads. The largest fragment, representing the posterior portion of the worm, is about 40 mm. long with a maximum thickness of 1·6 mm. at its posterior end. Judging from the state of development of the organs in the anterior segment of this fragment, and organs in the posterior segments of another fragment containing a head it is probable that an entire worm would reach about 70 mm. in length.

The head is top-shaped and has a maximum diameter across the posterior third of the suckers of 0·29 mm. The rostellum is well developed and is in all cases retracted into the scolex; it is from

0·2 to 0·212 mm. long with a maximum thickness of 0·093 to 0·102 mm. It carries a single row of rose-thorn shaped hooks (Fig. 12 a) which in the five heads numbered 15, 17, 15, 14, and 15 respectively. From the tip of the blade to the tip of the handle the

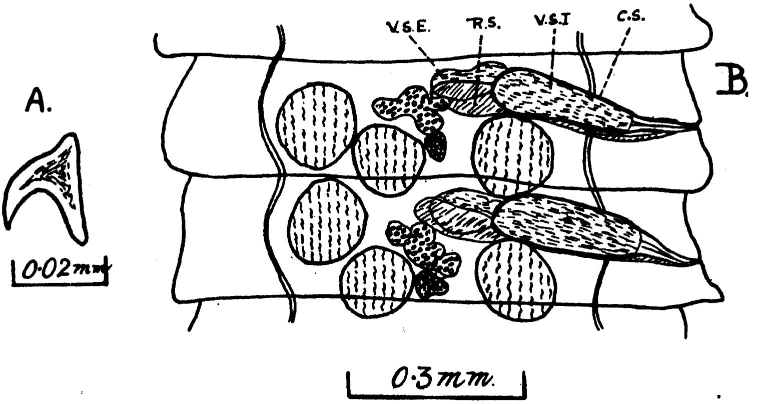


Fig. 12.—*Hymenolepis suricattae* sp. nov. Mature segment. (C.S. = Cirrus Sac; R.S. = Receptaculum Seminis; V.S.E. = Vesicula Seminalis externa; V.S.I. = Vesicula Seminalis Interna.)

size varied from 0·021 to 0·023 mm. and from the tip of the handle to the tip of the guard 0·019 to 0·02 mm. The four suckers are oval about 0·17 mm. long by 0·14 mm. across. The neck varies considerably in length, being from 1·4 to 3·5 mm. long by 0·16 to 0·174 mm. broad. The segments, of which there are about 400 to each strobila, are all broader than long. Those towards the anterior end and just showing the beginnings of the developing genitalie are 0·326 mm. wide and 0·08 mm. long; mature segments measure 1·13 to 1·2 mm. broad by 0·162 to 0·17 mm. long and ripe segments are 1·35 mm. broad and 0·225 mm. long. The genital pores are all unilateral and situated mostly just behind the middle of the segment.

The ventral excretory canals are situated 0·29 to 0·30 mm. from the aporal and poral margins respectively in mature segments and have a diameter of 0·006 mm. The dorsal excretory vessels were not observed. The longitudinal muscles consist of the usual two layers of fibre bundles.

The three testes are arranged in a flat triangle (Fig. 12 B), two being aporal and one poral in position; the central testis lies immediately posterior of the ovary and the outermost aporal testes is situated slightly anterior and lateral of this medial testis; they are slightly oval organs measuring in their mature state 0·145 to 0·17 mm. by 0·11 to 0·13 mm. The cirrus sac is relatively thin walled and club shaped and extends over the excretory canals to terminate anterior of the poral testes; it is from 0·34 to 0·36 mm. long by 0·072 to 0·098 mm. broad in mature segments. The cirrus is small and inconspicuous and the greater portion of the cirrus sac is filled by the large vesicula seminalis interna which is from 0·19 to 0·22 mm. long by 0·072 to 0·095 mm. in diameter. A large and slightly twisted vesicula seminalis externa extends to the ovarian field.

The ovary is centrally placed and is transversely elongated and lobed; it is 0·23 to 0·255 mm. broad by 0·09 to 0·115 mm. long. The yolk gland lies immediately posterior of the ovary; it may be rounded, oval or slightly lobed and has a diameter of about 0·075 mm. The vagina opens ventral of the cirrus sac and is provided with a voluminous reseptaculum seminis which increases in size posteriorwards but diminishes in size again in those segments where the eggs are maturing. The uterus is a transverse and sacculated bag which in mature segments fills practically the whole segment, squeezing the excretory canals against the lateral margins. The eggs have three envelopes, the thin shelled egg embryophore measures 0·024 mm. and the embryo 0·022 mm. in diameter respectively, and the hexacanth hooks 0·0084 mm.

Affinities.—Except for one doubtful record by Gaiger (1915) of a *Hymenolepis* sp. from a dog, this is the first record of a member of this genus from a carnivore; the nature of the hooks, belonging to group IV of Fuhrmann (1906), their number and size easily distinguishes this species from the known mammalian species of this genus. The shape of the hooks is very similar to that found in *H. petrodromi* Baer, 1933, from an Insectivore, but in this latter species the hooks number only ten and are much smaller; besides its testes are arranged in a transverse row. Similar types of hooks are also found in *H. globirostris* Baer, 1925, from Rodents and *H. uncispinosa* Joyeux and Baer, 1930, also from Rodents; in the former species however the hooks are fewer in number (12-14) and smaller (0·018 mm.) and in the latter fewer in number (12) and much larger (0·034 mm.).

Specific diagnosis.—Hymenolepidinae of medium size which may reach 70 mm. in length; head small and cone-shaped; suckers oval, hooks 14-17 in a single row, rose-thorn shaped and 0·021 to 0·023 mm. long from tip of blade to tip of handle; two aporal and one poral testes, arranged in a flat triangle; vesicula seminalis interna and externa large; cirrus sac crosses excretory canal and reaches poral testes; ovary central and lobed; uterus sacculated and fills practically whole segment in ripe segments. Eggs thin shelled.

Host: Suricata suricatta suricatta Erxl.

Location: Small intestine.

Locality: Fauresmith, Orange Free State.

Types in the Onderstepoort Helminthological Collection.

Fam. TAENIIDAE Ludwig, 1886.

Cladotaenia freani sp. nov.

The specimens of this species were submitted to this institute for determination by Mr. J. R. Frean, Government Veterinary Officer, at Potchefstroom, Transvaal. He obtained them from a Black Eagle

Hawk, shot at Victoria West, Cape Province. The material consisted of four strobilae with ripe segments but no scolices and three fragments with scolices; two of the latter still retained most of their hooks.

The four strobilae measured respectively 77, 98, 102, 107 mm. with a marimum thickness at about their maddle of 2·5 mm. The worms are considerably shrunken, so that when extended the length will be considerably more. The segments are at first broader than long; as the segments grow older they tend to become longer so that in segments where the sexual glands are disappearing they are more or less square; in older segments they become narrower and longer so that ripe segments may reach a length of 3·4 mm. by 1·3 mm. broad.

The scolex is dorso-ventrally flattened and has a transverse diameter of 0·3 mm. by 0·2 mm. thick (Fig. 13 A & B). The rostellum is prominent and 0·25 mm. broad by 0·16 mm. thick; it carries a double circlet of hooks; although all the hooks are not present it is possible to determine that there were 42 on the one scolex and 50 on the other. The anterior longer hooks are 0·031 to 0·035 mm. long from the tip of the blade to the end of the handle; the smaller are 0·024 to 0·028 mm. long between the same points (Fig. 13 C and D). In both the blade and handle are more or less on the same axis, and in the longer hooks the end of the handle is bent upwards and in the smaller hooks it is bent downwards. The four suckers are somewhat oval being 0·13 mm. long by 0·107 mm. across; in lateral view of the scolex they stand out prominently from the scolex itself. There is a short neck, about 0·46 mm. long by 0·27 mm. broad; the first segment is only about 0·035 mm. long

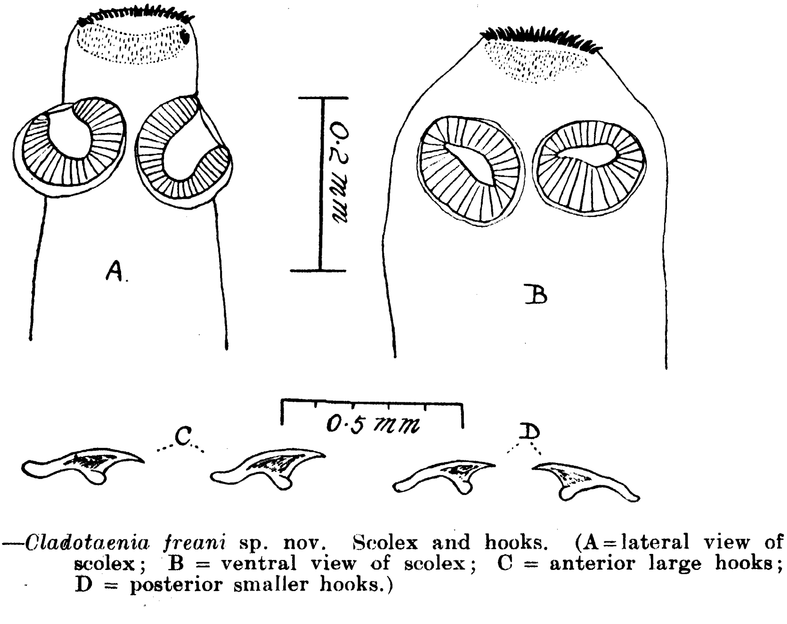


Fig. 13.—*Cladotaenia freani* sp. nov. Scolex and hooks. (A = lateral view of scolex; B = ventral view of scolex; C = anterior large hooks; D = posterior smaller hooks.)

by about 0·23 mm. broad. The excretory system consists of two pairs of longitudinal canals; the ventral canals are situated about 0·35 mm. from the edge of the segment and have a diameter of 0·04 to 0·09 mm. and are joined together by a transverse canal at the posterior end of each segment. The dorsal excretory canals are very small and have a diameter of only about 0·003 mm.; they are thick walled and are situated dorso-laterally of the ventral canals on their inner side. The genital canals pass between the excretory canals and ventral of the nerve cord.

The nerve cords lie lateral of the excretory canals and about 0·29 mm. from the edge of the segment.

The longitudinal muscles are well developed and form a single sheet about 0·055 mm. thick; it consists of numerous irregularly scattered muscle bundles, each bundle composed of from two to 40 fibres, about 0·003 to 0·004 mm. thick; the circular muscles are represented by a thin sheet just internal of the longitudinal muscles and separating the cortical from the medullary parenchyma. Transverse and dorso-ventral muscles appear to be very poorly developed. The subcuticular parenchyma carries numerous chalk bodies about 0·007 mm. in diameter.

The genital atria alternate irregularly and are situated in the anterior half of the segment, at about the junction of the second and third sevenths of the segment in mature segments. A deep genital atrium about 0·06 mm. deep joins the genital pore to the openings of the genital canals (Fig. 14). The cirrus sac is pyriform, muscular, about 0·13 mm. long with a maximum diameter of 0·07 mm. There are about 80 to 100 testes but in the contracted segments they are so closely packed that it was not possible to determine their number with certainty; they are arranged in two longitudinal fields bounded externally by the ventral excretory canals and internally by the field occupied by the female glands; they extend posteriorly at the sides of the ovary to the level of the yolk glands but do not pass inwards behind these glands; no testes are present in the field immediately anterior of the ovary; they form one or two layers, generally only one. The ovary consists of two lobed wings situated

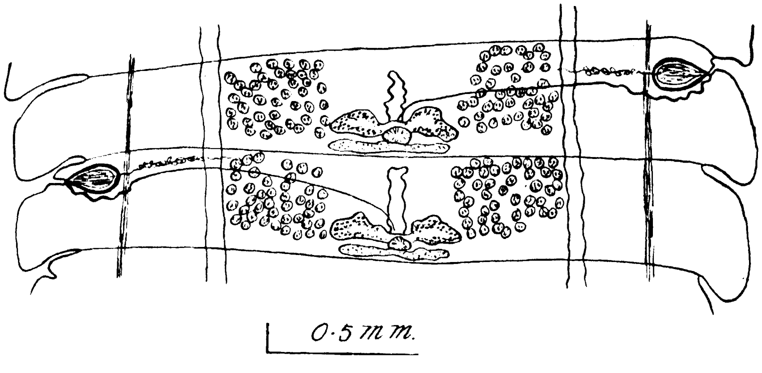


Fig. 14.—*Cladotaenia freani* sp. nov. Mature segment.

in the posterior and ventral portion of the segment; it is rather small, being in mature segments at most 0·38 mm. broad; the yolk gland is situated immediately behind and in the same plane as the ovary and is almost as wide as the ovary. The shell gland is rounded, situated behind the ovary but dorsal of the yolk gland. The vagina opens into the genital sinus on the same level but behind the orifice of the cirrus sac; it passes inwards forming a number of small convolutions and then forms an arch which passes into the shell gland in the middle of the isthmus joining the two ovarian wings; at its distal end it is widened to form a small reseptaculum seminis. The uterus (Fig. 15) consists of a medium stem with about 10 to 14 lateral branches. The median stem generally does not reach the anterior border, but segments where it does extend through the segment are not uncommon. The numerous eggs are slightly oval and smooth shelled, the shell being only 0·0015 mm. thick; the egg (embryophore) is 0·035 to 0·036 mm. long by 0·032 mm. across, the contained embryo has a diameter of 0·02 to 0·021 mm. of the hexacanth hooks are 0·007 mm. long.

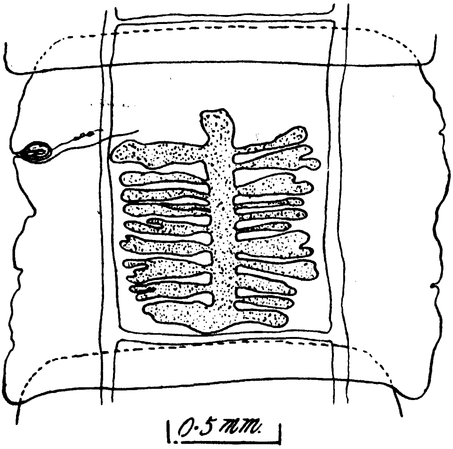


Fig. 15.—*Cladotaenia freani* sp. nov. Ripe segment showing uterus.

Affinities:—Up to the present four species of Taeniidae have been described from birds of prey (Accipitres) namely *Cladotaenia armigera* (Volz, 1900), *Cladotaenia cylindracea* (Bloch, 1782), *Taenia heteracantha*, Fuhrmann, 1906, and *Taenia s.l. hassalli* Fuhrmann, 1932. A description of the lastnamed is unfortunately not available to the writer so that it cannot be compared with the writer's species. *Taenia heteracantha* is characterised by having large hooks measuring 0·2 and 0·14 mm. long for the large and small hooks respectively. *Cladotaenia cylindracea* is very similar to the writer's species in that testes do not encroach into the ovarian field anterior to the ovary, but differs from it in that its testes pass posteriorly and meet behind the yolk glands, and because the median stem of the uterus in the writer's species may reach the anterior border of the segment. *C. armigera* differs in that its testes meet behind the female glands.

Specific diagnosis.—Taeniidae of small size, attaining a length of 10·7 cm. with a maximum breadth of 2·5 mm. Scolex small, rostellum with double crown of 42 to 50 small hooks, 0·031 to 0·035 mm. long for the larger and 0·024 to 0·028 mm. long for the smaller. Genital pores irregularly alternate at about the junction of 2nd and 3rd seventh of segment. About 80 to 100 testes in two longitudinal fields lateral of ovarian fields; no testes in fields anterior of ovaries; they do not extend backwards beyond anterior level of yolk glands. Ovaries relatively small and confined to area between testicular field. Uterus a median stem with 10-14 lateral branches; median stem generally does not extend to anterior border of segment, although it may occasionally do so. Eggs numerous; embryophore oval and and smooth shelled measuring 0·035 to 0·036 by 0·031 to 0·032 mm.

Host: Black Eagle Hawk. [? *Pteroaetus verreauxi* (Less).]

Location: Small intestine.

Locality: Victoria West, Cape Province.

Types in Onderstepoort Helminthological Collection.

Cladotaenia vulturi sp. nov.

Four fragments of this interesting parasite were available which, from the state of development of the internal organs, would appear to present portions of the same worm. In the oldest segments available the genital glands have just disappeared, so that if only one worm is represented then it must attain a length considerably longer than the total length of the fragments which total 80 mm., with a maximum breadth of 1·9 mm. The longest fragment is 38 mm. long and is provided with a scolex which unfortunately has a portion of its anterior face snipped off, so that the nature of the rostellum remains unknown. One small taenoid hook (?posterior), measuring 0·026 mm. in length, was adhering to the scolex, so that it appears legitimate to assume that the rostellum was armed. Unfortunately during the course of mounting the single hook was lost before a drawing of it was made. The remains of the scolex measure 0·4 mm. across and the somewhat circular suckers have a diameter of 0·2 mm. The neck is somehat contracted and is 0·5 mm. long.

From the toto-mount two large lateral ventral excretory vessels can be made out; in mature segments they have a diameter of 0·09 mm. and are united to each other by a large transverse duct at the posterior end of each segment; they were situated 0·26 to 0·29 mm. from the edge in mature segments.

The genital pores alternate irregularly and are situated in the anterior quarter of the segment at about the junction of the 1st and 2nd fifths; they are not raised on a genital papilla and they lead into a cup-shaped genital atrium about 0·05 mm. deep by 0·03 mm. across. The muscular cirrus sac is pyriform, 0·13 to 0·15 mm. long and 0·08 mm. maximum diameter; it does not reach the ventral excretory canal (Fig. 16 A & B). The vas deferens after crossing the excretory canal becomes intricately coiled and extends straight

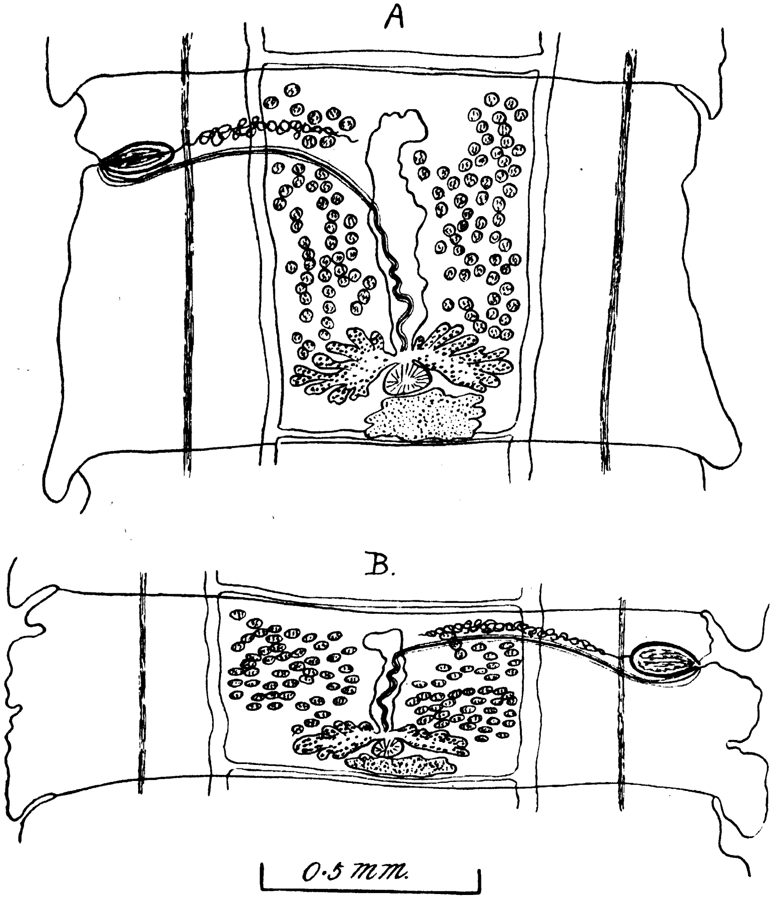


Fig. 16.—*Cladotaenia vulturi* sp. nov. Mature segments. (A = extended segments; B = contracted segment.)

inwards almost to the midline of the segment. The testes, of which there are from 80 to 110, are all situated anterior of the ovary, none being present on the lateral or posterior sides of this organ; in extended segments (Fig. 16, A) they ar confined to the field limited by the width of the ovaries, the central or uterine area being devoid of testes; as in these extended segments the ventral excretory canals just skirt the lateral limits of the ovaries and as the testes do not extend lateral of these canals it will be seen that the testes become confined only to the field anterior of the ovaries. In segments, however, which are contracted (Fig. 16 B) the excretory canals are pushed outwards and the same applies to the testes; in these segments testes are present in the areas lateral of the ovarian fields, but no testes are found lateral or posterior of the ovaries themselves. This distribution of the testes is a characteristic which is constant in all the segments and easily distinguishes this species from the preceeding species.

The vagina opens into the genital atrium just behind the cirrus sac; it forms a gentle curve round the cirrus sac and then extends inwards somewhat parellel to the course occupied by the vas deferens; on reaching the midline it bends backwards and after being thrown into a few prominently staining loops it disappears in the isthmus of the ovaries after having formed a small reseptaculum seminis. The two-winged ovaries are large and multilobed and are about 0·44 mm. wide by 0·12 mm. long; the yolk gland is situated behind the ovary and is about 0·29 mm. broad by 0·085 mm. long; a small rounded to oval shell gland measuring 0·04 to 0·06 mm. across is intercalated between the ovaries and shell gland. The median stem of the uterus extends forwards to the level of the genital pore or almost to the anterior edge of the segment. No fully developed uteri are present, but from the partially developed uteri it would appear that there are about ten lateral branches on either side of the median stem.

Affinities: The small size of the hooks distinguished this species from *Taenia heteracantha* Fuhrmann (1906) and the distribution of the testes distinguishes it from the species described above and from the species *Cladotaenia armigera* (Volz, 1900) and *C. cylindracea* (Bloch, 1782).

Specific diagnosis.—Taeniidae of small size provided with a small scolex and small rostellar hooks, genital atria irregularly alternate and in anterior fifth of segment. Testes number 80 to 110, all confined to area anterior of ovaries and do not cross the excretory canals; central uterine area of segment devoid of testes. Vagina passes down centre of segment and forms few prominent coils; ovaries multilobed and large. Uterus with apparently about 10 lateral branches on either side.

Host: " Vulture ".

Lication: Small intestine.

Locality: Kruger National Park, Transvaal.

Type slide in Onderstepoort Helminthological Collection.

Since submitting this paper to the press the writer has seen a paper by Yamaguti (" Studies on the Helminth Fauna of Japan. Part 6. Cestodes of Birds I." *Jap. Jl. of Zool.* Vol. 6, pp. 183-232. Tokio, 1935) wherein a new species—*Cladotaena circi*—is described from *Circus aeruginosus aeruginosus* (L.). In addition a new genus—*Paracladotaenia*—is created for the reception of *P. accipitris* sp. nov. from *Accipiter virgatus gularis* (Temm. and Schl.). The former species differs from *C. freani* sp. nov., described above, in having smaller hooks (0·018 and 0·024 mm.) and in that the testes meet behind the female glands. This latter character also distinguishes Yamaguti's species from *C. vulturi* sp. nov. The genus *Paracladotaenia* differs principally from the genus *Cladotaenia* in that the testes are arranged in two separate groups and not united behind the female glands; the uterus extends through the length of the segment; a vesicula seminalis interna is present, and rostellar hooks are absent. The species *C. freani* sp. nov. and *C. vulturi* sp. nov, resemble *P. accipitris* in having two lateral groups of testes and the uterus in some cases extends through the length of the segment. Should additional material of Yamaguti's species become available for examination and this material showed that rostellar hooks are normally present in this species and had been lost in the type material, then the writer would have no hesitation in assigning his two species to the genus *Paracladotaenia* rather than to the genus *Cladotaenia.*

In the above paper Yamaguti also refers to two species of *Cladotaenia* (*C. feuta* and *C faria*) described by Meggitt (1933), whose descriptions the writer has not been able to consult. The former species has no rostellar hooks and has less than 100 testes; the latter species has 20 rostellar hooks, 0·006 to 0·007 mm. long, and also has about 100 testes. As Yamaguti compares these two species with his species *Cladotaenia circi*, the writer infers that in Meggitt's species the testes are not arranged in two separate groups.

SUMMARY.

The writer here describes eight new species of cestodes, three from mammals and five from birds; the mammalian species are *Inermicapsifer leporis* sp. n. from a hare, *Catenotaenia geosciuri* sp. n. from a suricat. The avian species are *Raillietina* (*Skrjabinia*) *deweti* sp. n. from a guinea fowl, *Schistometra korhaani* sp. n. from a bustard, *Onderstepoortia taeniaeformis* gen. and sp. n. from a stone curlew, *Cladotaenia freani* sp. n. and *Cladotaenia vulturi* from birds of prey.

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South African Helminths.—Part IV.

Cestodes from Columbiformes.

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In 1923 Joyeux recorded four species of *Raillietina*, one species of *Aporina*, two species of *Cotugnia* and two species of *Hymenolepis* from five kinds of African pigeons. Le Roux (1927) found two species of *Raillietina* in domestic pigeons from Natal, namely *Raill. (Fuhrm.) crassula* (Rud., 1819) and *Raill. (Raill.) crassula* (Rud.) of Clerc, 1906, which species had been renamed *Raill. (Raill.) clerci* by Fuhrmann (1920).

According to Fuhrmann (1932), 41 species and varieties of Cestodes have been recorded from Columbiformes. Since this date four further species of Raillietina have been described, namely *Raill. (Raill.) idiogenoides* Baer, 1933, from *Vinago delalandei*, Rhodesia; *Raill. (Raill.) perplexa* Johri, 1933, from *Columba livia intermedia*, India; *Raill. (Raill.) taiwanensis* Yamaguti, 1935, from *Columba livia domestica*, Formosa; and *Raill. (Raill.) vogeli* Hilmy, 1936 from *Vinago calva sharpii*, Liberia. In addition descriptions of five further species and one sub-species of *Cotugnia* have appeared, namely *C. intermedia* Johri, 1934, and *C. noctua* Johri, 1934, from *Columba livia intermedia*, India; *C. bahli* Johri, 1934, from *Turtur suratensis*, India; *C. meggetti*, Yamaguti, 1935 and *C. taiwanensis* Yamaguti, 1935, from *Columba livia domestica*, Formosa, and *C. polycantha oligorchis* Joy., Baer and Martin, 1936 from *Stigmatopelia senegalensis*. Also descriptions of two species of *Killigrewia* namely *K. oenopopeliae* Yamaguti, 1935, from *Oenopopeliae tranquebarica humilis*, and *K. streptopeliae* Yamaguti, 1935, from *Streptopelia chinenensis formosa*, both from Formosa. Two earlier described species of this genus must be added to these, namely *K. frivola* Meggitt, 1927, which Meggitt described from an unknown host from Egypt but which Johri (1934) has obtained from *Columba livia intermedia*, from India, and *K. pamelae* Meggitt, 1927, from *Turturoena sharpii* from Egypt. And finally one species of *Hymenolepis*, *H. streptopeliae* Joyeux and Baer, 1935, from *Streptopelia orientalis orientalis* from Indo-China. This brings the total of cestode species and sub-species from Columbiformes up to date, including the four new species described below, to fifty-nine.

During the last few years the writer has been able to examine materials originating from four kinds of pigeons, namely Domestic pigeons, Green Fruit pigeons, Laughing doves and Namaqua doves; this material was found to comprise five species of *Raillietina*, three of which the writer considers as new and one new species of *Hymenolepis*.

The writer did not obtain any examples of *Railletina (Raill.) clerci* Fuhrmann from domestic pigeons, but he obtained examples of a cestode which has some similarity to the above species but which the writer considers to be new; whether this material is identical with that which le Roux (1927) identified as *Davainea crassula* (Rud., 1819, Clerc 1906 [=*Railletina (Raill.) clerci*] the writer is not in a position to say as le Roux gives no data concerning this species and his material is not available for comparison.

Raillietina (Fuhrmanetta) crassula (Rud., 1819).

This species was recovered from Domestic pigeons from three localities, namely Onderstepoort, City of Pretoria and Grahamstown. The writer's materials show the following characteristics which in some aspects differ from the descriptions given by Fuhrmann (1909) and Neveu Lemaire (1936). In the following the figures between brackets are those given by these two authors.

The length of the strobilae varies from 199 to 442 m.m. (250 to 400 mm.) and the breadth is never more than 2 mm. (4 mm.); the hooks number from 120 to 140 (70), the majority of scolices carrying about 120 hooks; the length of those of the anterior row was found to be from 0·017 to 0·021 (0·02 mm.) the usual length being 0·02 mm.; those of the posterior row varied in length from 0·015 to 0·016 mm. the usual length being 0·016 mm. The breadth of the scolex is from 0·246 to 0·38 mm. (0·23 mm.) and the rostellum measures 0·07 to 0·09 mm.; the armed suckers are round to slightly oval, 0·087 to 0·91 mm. to 0·096 by 0·126 mm. (0 09 mm.). The cirrus sac is from 0·078 to 0 145 mm. long (0·1 mm.) and the testes vary in number from 35 to 61 (30 to 40), arranged laterally and behind the female glands, about 25 to 40 being aporal; in some strobilae they cross the excretory canals. The ovary is multilobate and poral in position. Each ripe segment carries about 300 egg capsules which cross the excretory canals and each capsule has from two to five eggs (3-4).

Hosts: *Columba livia domestica.*

Location: Small intestine.

Locality: Transvaal and Cape Eastern Province.

In addition to the above-described materials two specimens of a nearly related, if not cospecific, cestode were obtained from a Laughing dove [*Stigmatopelia senegalensis aequatorialis* (Erl.)] shot in the Onderstepoort grounds. These specimens agree in all essentials with those described above except that they are much smaller, reaching only 84 mm. in length by 1·2 mm. broad, and the end segments contain much fewer egg capsules (65 to 75). Whether these differences are of specific importane or whether they are due to different host reactions, the writer cannot say; however, in the meantime the writer is referring these specimens to the above species and considering them to be a minor variety.

Raillietina (Raillietina) columbiella sp. nov.

This species was present in fair numbers in five of six domestic pigeons shot at Onderstepoort; on two occasions the host also harboured *Raillietina (Fuhrmanetta) crassula* (Rud.). According to the state of contraction the total length of complete strobilae varies from 34 to 67 mm. and the breadth by 1·5 to 2 mm.; the shorter strobilae have a lanceolate appearance. In all, a striking feature is the extreme shortness of the segments compared with their breadth; only the hindmost segments show a tendency to become elongate but even in these their breadth is always greater than their length.

The scolex is relatively small and has a transverse diameter of 0·188 to 0·27 mm.; the four suckers are slightly oval and measure 0·058 by 0 07 mm. to 0·08 by 0·09 mm.; they are armed with about 10 rows of numerous hooks, the longest measuring 0·01 mm. long. The rostellum has a thickness of 0·12 to 0·18 mm. and carries about 200 typically hammer-shaped hooks arranged in two rows; those ot the anterior row are from 0·019 to 0·022 mm. long and those of the posterior row 0·016 to 0·018. The base of the rostellum is covered by numerous very minute spines.

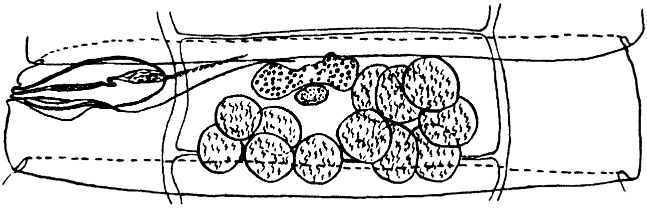

Fig. 1.—*Raill.* (*Raill.*) *columbiella* sp. nov. Mature segment.

The neck may reach a length of 1·75 mm. by 0·2 mm. broad. The genital pores are unilateral and situated in the anterior third of the segment. The cirruc sac is relatively long and almost reaches the excretory canal but in some cases may partially lie over it; it is from 0·16 to 0·23 mm. long by 0·05 to 0·087 mm. thick; its wall is not very muscular (Fig. 1); the cirrus sac is armed with minute spines which appear to be limited to its distal half. A vesicula seminalis interna is present and the vas deferens is much coiled. The testes number from 11 to 13, are round and have a diameter of 0·063 to 0·077 mm. The ovary is two lobed and slightly crenated and occupies about the central fifth of each segment; the yolk gland is oval and about one-third the size of the ovary. There are about 50 to 80 egg capsules to each segment and these cross the excretory canals laterally; each capsule has from four to eight eggs.

Affinities.—*Raillietina (Raill.) taiwanensis* Yamaguti, 1935, possesses circa 200 rostellar hooks, about 0·02 mm. long and has testes approaching in number those of the species described above. *R. (R.) insignis* (Steudner, 1877) and *R. (R.) nagpurensis* Moghe, 1925 also have rostellar hooks of about the same size and number but have more testes (20 to 25 and 19 to 22 respectively). The writer's species can be distinguished from Yamaguti's species in that the latter has no spiny collar round the base of the rostellum and the cirrus sac is much smaller.

Raill. (R.) clerci Fuhrmann, differs from the writer's species in having numerous rostellar hooks (400?) which are much smaller (0·01 mm.); it has about 20 testes and the egg capsules do not cross the excretory canals.

Specific diagnosis.—Davaineidae which may reach a length of about 70 mm. by 2 mm. broad; and with segments much broader than long. Rostellum carries circa 200 hooks in two rows, those of the anterior row being 0·019 to 0·022 mm. long and those of the posterior row 0·016 to 0·018 mm. long. Suckers armed and base of rostellum covered with numerous minute spines. Genital pores unilateral. Cirrus sac up to 0·23 mm. in length and reaches the excretory canals, Vesicula seminalis interna present. Testes 11 to 13. Ovary two-lobed. Eggcapsules 50 to 80, crossing excretory canals and each contains four to eight eggs.

Host : *Columba livia domestica.*

Location : Small intestine.

Locality : Onderstepoort, Transvaal.

Types in the Onderstepoort Helminthological Collection.

Raillietina (*Raillietina*) *johri* sp. n.

Syn. *Raillietina* (*Raill.*) *polychalix* Kotlan ,1920, Johri, 1934.

Not *Raillietina* (*Raill.*) *polychalix* Kotlan 1920.

In 1934 Johri reported the species *Raillietina (Raillietina) polychalix* 'Kotlan, 1920, from *Columba livia domestica* and *Psittacula krameri manillensis*; the material from the former host differed from the latter in having a greater number of rostellar hooks (324 against 190), which are smaller (0·011 and 0·014 mm. against 0·0135 and 0·019 mm.), fewer testes (eight to nine against nine to eleven), larger cirrus sac (0·125 to 0·13 mm. against 0·061 mm.) and fewer egg capsules (30 to 40 against 48). Johri considers these differences not to be of sufficient weight to warrant the separation of these materials into different species. The writer, however, does not support this view and he considers that the great disparity in the number of rostellar hooks and sizes of the cirrus sac definitely show that these materials are not cospecific. Besides it is highly improbable that the same species of cestode would occur in such widely differing groups (Columbiformes and Psittaciformes). The writer has much pleasure in naming this species after its discoverer.

Affinities.—This species appears to be closely related to if not cospecific with *Raillietina (Raill.) spiralis* Baczynska, 1914, from *Columba* sp. New Guinea. From the data available Baczynska's species would appear to have the same number of rostellar hooks (300) which are only slightly longer (0·0156 mm.), the scolex has about the same size (0·224 mm.), the cirrus sac is only slightly smaller (0·101 mm), the testes are slightly fewer (six to seven) and the egg capsules each contain four to six eggs. However, since the writer is not able to make a personal comparison between Johri's and Baczynska's materials he considers it advisable in the meantime to treat those materials as representing distinct species.

Host : Columba livia domestica.

Locality : Lucknow, India.

Raillietina (Raillietina) vinagoi sp. n.

This species, represented by several complete specimens from two Green Pigeons, has a total length of 115 to 123 mm. by 1·5 to 2 mm. broad.

The scolex measures 0·48 to 0·52 mm. across. The rostellum measures 0·085 mm. across and carries a double row of small hammer-shaped hooks; these number about 350. Their sizes are 0·017 and 0·014 for those of the anterior and posterior rows respectively. The suckers are somewhat rounded and armed and have a diameter of 0·075 mm. The neck is about 1·0 mm. long by 0·116 mm. broad.

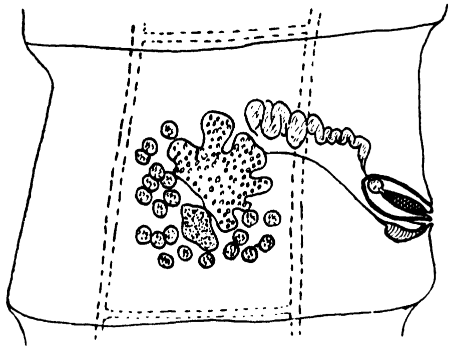

Fig. 2.—*Raill.* (*Raill.*) *vinagoi* sp. nov. Mature segment.

The genital pores are unilateral and in contracted segments they are situated in the anterior third of the segment; in well extended segments their position is at the junction of the second and last thirds of the segments (Fig. 2). The cirrus sac is large and prominent and varies in shape from oval to flask-shaped; it is from 0·165 to 0·18 mm. long by 0·093 to 0·102 mm. in diameter. A vesicula seminalis interna is present and the vas deferens is densely coiled. There are generally from 18 to 24 testes to each segment of which five to eight are poral in position; exceptionally, however, as many as 30 testes are present; in some segments they are arranged in a semi-circle, lateral and behind the female glands whereas in others they form two lateral groups separated by the female glands. The vagina opens behind the cirrus sac and enters the genital atrium at an angle; its distal portion is pear-shaped with a relatively large lumen and is surrounded by circular muscle fibres which give this portion a hooped appearance. The ovary is centrally placed and multibolebd and large and measures about 0·25 mm. long by 0·2 mm. broad; the vitelline gland is roughly oval measuring about 0·12 mm. long by 0·075 mm. broad. The egg capsules fill the whole segment, but do not extend beyond the excretory canals; each segment carries 50 to 55 capsules and each capsule has three to ten eggs.

Affinities.—The only *Raillietina (Raillietina)* sp. from Columbiformes having 300 or more rostellar hooks which are more than 0·01 mm. but less than 0·02 mm. long, and possessing more than 20 testes is *Raillietina (Raill.) perplexa* Johri, 1933 and the species described above; Johri's species differs from the writer's in possessing a larger scolex (0·35 mm.), larger rostellum (0·183 mm.) with collar of numerous spines, the cirrus sac is only about half the size (0·087 to 0·97 mm.), and the testes are relatively more (26 to 28).

Specific Diagnosis.—Davaineidae possessing about 350 rostellar hooks measuring 0·017 and ·014 mm.; genital pores unilateral; cirrus sac large, oval or flask-shaped up to 0·18 mm. long; vesicula seminalis interna present; testes usually 18 to 24 in number, exceptionally reaching 30; they either form a semi-circle round female glands or are arranged in two groups with five to eight testes poral. Distal portion of vagina large, muscular and pear-shaped with large lumen; ovary multilobed; 50 to 55 egg capsules to each segment, not passing over excretory ducts; each capsule with three to ten eggs.

Host: Vinago delalandei delalandei (Bp.).

Location: Small intestine.

Locality: Transvaal and Natal.

Types in the Onderstepoort Helminthological Collection.

Raillietina (Raillietina) delalandei sp. nov.

This delicate cestode reaches a length of 78 mm. by 0·36 to 0·48 mm. broad; the scolex has a breadth of 0·16 mm. and its rostellum has a diameter of 0·08 to 0·085 mm. The hooks are numerous and arranged in two rows; their total number appears to be between 600 and 700 and their length is 0·01 and 0·011 mm. The suckers are rounded and about 0·07 mm. across and each is heavily armed, the largest spines reaching 0·014 mm. in length. The neck varies in length from 0·58 to 0·76 mm. and is about 0·085 mm. broad.

The genital pores are unilateral and their position varies according to the state of contraction of the segment; generally they are situated just anterior to the middle of the segments (Fig. 3). The cirrus sac and distal portion of the vagina are similar to those seen in the preceding species except that they are smaller; the cirrus sac is from 0·11 to 0·12 mm. long by 0·063 mm. wide and the cirrus is spined. There are 12 to 15 rounded testes of which about six are poral and the remainder aporal in position; sometimes, especially in young segments, these two groups are joined to each other by one or two testes situated behind the female glands. The ovary consists of two almost circular wings, each about 0·06 mm. in diameters, and is centrally placed; their outlines is generally smooth but they may be slightly lobate. The circular to oval yolk gland lies immediately behind the ovary between its two wings; its diameter is from 0·06 to 0·072 mm. There are 20 to 25 egg capsules to each segment, each containing six to ten eggs; they do not extend beyond the excretory canals.

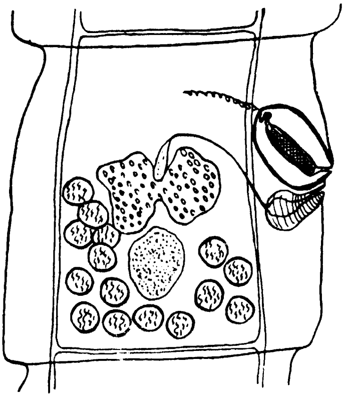

Fig. 3.—*Raill.* (*Raill.*) *delalandei* sp. nov. Young segment.

Affinities.—The size of the rostellar and sucker hooks and number of testes allies this species to *Raill. (R.) gendrei* Joyeux, 1923, from *Vinago calva*, French Guinea; it, however, differs from this species in having about three times as many rostellar hooks, only about one-fifth the number of egg capsules per segment and the cirrus sac is smaller. It appears to have its closest relatives in *Raill. (R.) vinagoi* sp. nov. described above and *Raill. (R.) clerci* Fuhrmann, 1920. It is easily distinguished from the former species by its smaller size and greater number of rostellar hooks which are much smaller; it differs from the latter species by its much smaller size, smaller spines on the suckers and much smaller cirrus sac.

Specific Diagnosis.—Davaineidae reaching a length of at least 78 mm. and provided with a rostellum carrying numerous (600 to 700) small hooks, 0·01 to 0·011 mm. long and arranged in two rows; suckers armed with large spines reaching 0·014 mm. in length. Genital pores unilateral, generally just in front of middle of segment; 12 to 15 testes arranged in a poral group of about six and rest aporal;

in young segments testes also behind female glands; cirrus sac 0·11 to 0·12 mm. long; 20 to 25 egg capsules to each ripe segment, each containing six to ten eggs; they do not extend beyond the excretory canals.

Host: Vinago delalandei delalandei (Bp.).

Location: Small intestine.

Locality: Natal South Africa.

Types in the Onderstepoort Helminthological Collection.

Raillietina (Raillietina) vogeli Helmy, 1936.

Several fragments of this species were available; these consisted of two scolices with portions of strobila and several portions with mature to ripe segments. The one scolex appears to possess all its rostellar hooks, but the other has lost them all; in both cases no hooks are present on the suckers.

The rostellar hooks number between 150 and 200 and are arranged in two rows; they measure 0·035 and 0·037 mm. in length. The scolices have a transverse diameter of 0·458 and 0·47 mm. and the suckers are rounded and measure from 0·165 to 0·174 mm. in diameter. The neck is about 1·5 mm. long by about 0·29 mm. broad.

The genital pores are unilateral and are situated in the anterior third of the segment. There are from 16 to 22 testes, of which four to six are poral in position; generally the poral and aporal testes form two separated groups lateral of the female glands but in some segments these groups may be united by one or two testes posterior of the female glands. The ovary is somewhat centrally placed and rounded and the vitelline gland is found behind it somewhat towards the aporal side. There are from 70 to 90 egg capsules, each containing three to six eggs.

Host: Vinago delalandei delalandei (Bp.).

Location: Small intestine.

Locality: Natal South Africa.

This species has been referred to Helmy's species because of the similarity in the size and number of rostellar hooks and because the material was collected from an African green fruit pigeon of the genus *Vinago*. Helmy was unfortunately not able to give an account of the male and female glands as his material was immature. The writer's material appears to differ from Helmy's in possessing a larger scolex and rostellum.

The only other species of *Raillietina* (*Raill.*) from Columbiformes whose hooks approach in size those of the writer's material is *R. (R.) fuhrmanni* (Southwell, 1922); the hooks of this species are, however, smaller (0·025 to 0·03 mm.), less in number (110) and the testes are also fewer (about 12).

Hymenolepis oena sp. nov.

This species was recovered from three of the Namaqua pigeons shot in the Onderstepoort grounds; one of the pigeons harboured four specimens. The worms vary in length from 76 to 94 mm. with a maximum breadth of 1·6 to 1·9 mm. towards their posterior ends. All the segments are much broader than long, mature segments reaching a length of 0·22 mm. by 0·89 mm. broad; ripe segments reach a length of 0·34 mm. by nearly 2 mm. broad.

The scolex (Fig. 4A) is small and somewhat spindle-shaped and measures from 0·10 to 0·24 across, and the four unarmed suckers are slightly oval being 0·086 to 0·09 mm. across by 0·1 to 0·12 mm. long. The rostellum is partially retracted in all the specimens but the extruded portion has the shape of a truncated cone with a basal thickness of 0·098 to 0·108 mm. and an apical diameter of 0·035 mm.; the rostellar sac is large and extends beyond the posterior level of the suckers being about 0·3 mm. long. There are 10 rostellar hooks arranged in a single circle; they are from 0·041 to 0·045 mm. long, and the blade, which has a length of about 0·015 mm., lies in a straight line with the handle (Fig. 4B). The neck varies in length from 0·58 to 0·75 mm. by 0·087 to 0·1 mm. broad.

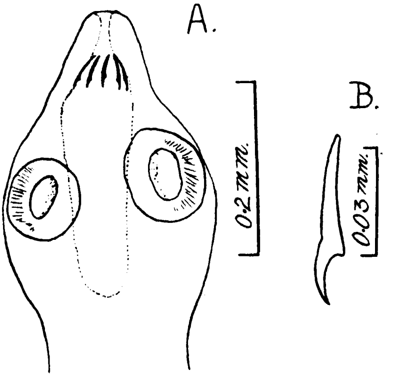

Fig. 4.—*Hymenolepis oenai* sp. nov. A. Scolex. B. Rostellar hook.

The genital pores are unilateral and are situated in the anterior quarter of the segment; they are not prominent. The cirrus sac is tubular to club-shaped, extends across the excretory canals and may pass slightly beyond the poral testis; it is from 0·2 to 0·25 mm. long with a maximum thickness in its proximal third of 0·072 mm. The cirrus appears to be unarmed and has a thickness of 0·012 to 0·015 mm. and reaches a length of 0·06 mm. when extruded. The three testes are arranged in a triangle, one poral and two aporal; the anterior aporal testis is situated slightly nearer the midline and is also generally smaller than that behind it; the poral testis is generally rounded with a diameter of 0·077 to 0·08 mm. while the aporal testes are generally oval, measuring about 0·1 by 0·05 mm. and 0·08 by 0·035 mm. for the posterior and anterior testes respectively. The ovary is centrally placed and at first consists of two wings forming an open and inverted V; as it matures it becomes slightly lobulated and assumes an irregularly rounded appearance when it measures about 0·15 mm. across. The yolk gland is an irregular oval, 0·05 to 0·056 mm. across, and lies between the two ovarian wings. The whole of the vagina is thin walled and in the older segments, especially those in which the genital glands have disappeared, its inner portion becomes very much enlarged to form a large receptaculum seminis reaching a length of 0·3 mm. by 0·15 mm. thick. The uterus arises as a transverse tube ventral of the ovary; as it enlarges it becomes sacculated and in ripe segments it fills almost the whole segment up to its lateral margins. The numerous eggs have three shells of which the middle is thickened to form an egg-shell; the outer shell is irregularly shaped but when rounded has a diameter of 0·045 to 0·048 mm.; the middle shell has a diameter of 0·03 to 0·033 mm.; the innermost shell closely surrounds the rounded embryo which measures 0·015 mm.

Affinities.—Ons species of this genus, namely *H. columbia* Fuhrm., 1909, has been described from this host, but the nature of the scolex and hooks of this species are quite different from those described above. The shape of the hooks shows some similarity to those of *H. serrata* Fuhrm., 1906 and *H. streptopeliae* Joyeux & Baer, 1935, in the former species the hooks are fewer (8) and their size is more than twice that of the writer's species, and in the latter the strobila is much longer and wider and the hooks are smaller (0·03 mm.).

Specific Diagnosis.—Hymenolepidinae which may reach a length of about 100 mm. by 2 mm. broad. All sgments much broader than long. Scolex spindle-shaped and small. Rostellum well developed with large rostellar sac extending posterior of level of suckers. Ten rostellar hooks, 0·041 to 0·045 mm. long, the handle and blade forming almost a straight line. Genital pores in anterior quarter of segment. One poral and two aporal testes. Cirrus sac tubular to club-shaped and may extend beyond poral testis. Ovary median, lobulate. Uterus fills up whole segment. Eggs three shelled.

Host: Oena capensis (L.).

Location: Small intestine.

Locality: Onderstepoort, Transvaal.

Types in the Onderstepoort Helminthological Collection.

SUMMARY.

The writer records the following species from four species of South African pigeons: *Raillietina (Fuhrmanetta) crassula* (Rud. 1819) from *Columba livia domestica* and *Stigmatopelia senegalensis aequatorialis. Raillietina (Raillietina) columbiella* sp. nov. from *Columba livia domestica; Railliteina (Raillietina) vinagoi*, sp. nov., *Raillietina (Raillietina) delalandei* sp. nov. and *Raillietina (Railliteina) vogeli* Hilmy, 1936 from *Vinago delalandei delalandei;* and *Hymenolepis ~~oenai~~* sp. nov. from *Oena capensis.*

In addition the species identified by Johri (1934) as *Raillietina (Raillietina) polychalix* Koltan, 1920 from an Indian domestic pigeon is considered by the writer as a new species and is named *Raillietina (Raillietina) johri.*

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South African Helminths.—Part V.

Some Avian and Mammalian Helminths.

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In the ensuing pages some helminths are described and discussed which were recovered from six species of birds and three species of mammals. Of particular interest is the multiple infection encountered in the Reed Cormorants and Giant Bustard; in one case of the former seven different kinds of worms were present, namely *Prosthogonimus cuneatus* (Rud., 1809), *Harvardia sandgroundi* Baer, 1932 and *Parphostomum radiatum* Duj., 1845 (Trematoda); *Ligula intestinalis* Linn., 1758, *Paradilepsis delachauxi* (Fuhrm., 1909) and *Hymenolepis cormoranti* sp. n. (Cestoda); and the nematode *Contracaecum carlislei* sp. nov. The Giant Bustard harboured eight different worm species, the majority of which appear to be new to science; these helminths were *Schistometra conoideis* (Bloch, 1782), *Schistometra* sp., *Idiogenes kori*, sp. nov. and *Idiogenes kolbei* sp. nov. (Cestoda) and *Subulura otidis* sp. nov., *Acuaria semei* sp. nov., *Histiocephalus choriotidis* sp. nov. and two immature female specimens of a *Habronema* sp. (Nematoda).

Class **TREMATODA.**

Prosthogonimus cuneatus (Rud., 1809).

On opening up the cloaca of a Reed Cormorant under water a single specimen of this species floated out into the water; in consequence it is not possible to say whether its location was the bursa fabricii or the cloaca.

The species is tentatively referred to the above species because from the mounted specimen (Fig. 1) no character was observed by which it could be differentiated from Rudolph's species. In the unpressed condition the total length is 2·5 mm. and the maximum breadth 1·7 mm., the oral sucker has a diameter of 0·31 mm. and the ventral sucker measures 0·68 mm. across. The much convoluted uterus is packed with numerous eggs of which about half are brownish and appear to be mature.

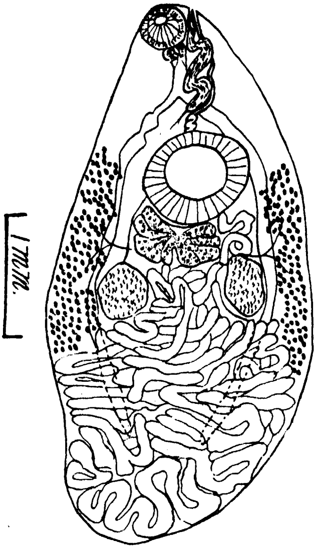


Fig. 1.—*Prosthogonimus cuneatus.* Ventral view of toto mount.

As far as the writer could ascertain this is the first record of a member of this genus from the Union.

Host: Microcarbo africana africanoides (A. Smith).

Lication: Cloaca (? Bursa fabricii).

Locality: Pretoria district.

Class **CESTODA.**

Ligula intestinalis Linneus, 1758

Two of three Reed Cormorants yielded four and two adult specimens respectively; they were allowed to die in cold water and were then fixed in cold 70 per cent. alcohol-glycerine with slight stretching; the two largest specimens measured 225 and 240 mm. in length with a maximum breadth of 5 and 5·5 mm. respectively. The anterior portion for a length of 25 to 35 mm. is very muscular and its lateral margins are provided with about 45 serrations on either side. These help to keep the worm in position and this portion may be compared to a metascolex, in that, although a scolex is present, it and its bothridial grooves are so small that it is doubtful whether they play any part as an organ for attachment. In transverse sections of the " metascolex " it is seen that the musculature is very well developed consisting of numerous bundles of longitudinal muscles arranged in 7 to 10 layers separated by sheets of transverse muscles; these sheets run more or less parellel to each other but they may join up to others immediately adjacent; the individual bundles are for the most part separated from each other by bundles of well developed dorso-ventral muscles. This extensive development of these muscles has caused the medulla to be represented by only a thin layer of parenchyma. Genital organs are present only in the posterior fifth of the "metascolex" and here two tocostomes, one in front of the other, are present opposite each lateral serration. In the remaining portion of the strobila, which becomes narrower posteriorly, the musculature is arranged somewhat similarly except that its degree of development decreases, until in the posteriormost portion bundles appear to be absent and only isolated fibres are found.

The genitalia are similar to those described by Baer (1933). The eggs are operculate and measure 0·052 to 0·06 by 0·047 to 0·05 mm.

Host: *Micracarbo africana africanoides* (A. Smith).

Location: Small intestine.

Locality: Pretoria district.

The writer is tentatively referring to this species some plerocercoids recovered from the body cavity of a fresh water fish, *Engraulicypris whitei*—collected from the Hartebeestpoort Dam by Miss Doreen Stewart of the Zoological Department, University of the Witwatersrand and placed at the writer's disposal for determination. These larvae vary in length from 20 to 30 mm. with a maximum breadth of 4 mm. Developing genitalia are present in their posterior two-thirds.

Raillietina (S.L.) thryonomysi sp. nov.

This species, collected from a Cane Rat, was represented by about half a dozen specimens all of which were unfortunately immature; they were from 9·5 to 16 mm. long with a maximum thickness of 0·5 to 0·6 mm. and consisted of a head, neck and from 64 to 114 segments.

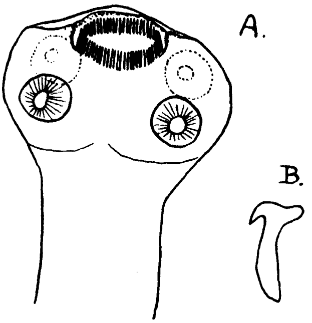


Fig. 2.—*Raillietina* (*S.L.*) *thryonomysi* sp. nov. A = scolex; B = hook.

The scolex (Fig. 2A) is prominent and measures from 0·42 to 0·48 mm. across; the suckers are rounded and unarmed and have a diameter of 0·115 to 0·12 mm. The rostellum is retracted in all cases and measures 0·18 to 0·192 mm. across; it carries a double row of about 100 typical hooks (Fig. 2B), the hooks of the anterior and posterior rows measuring respectively 0·04 and 0·036 mm. in length. The base of the rostellum is covered by innumerable small spines; the fact that these spines and rostellar hooks are present on all the scolices makes the absence of spines from the suckers appear to be a normal occurrence and not due to their loss. The neck is fairly long and may reach 1·6 mm. in length; its breadth varies from 0·228 to 0·252 mm.

The genital pores are unilateral and situated in the anterior quarter of the segment. The cirrus sac is somewhat pear-shaped and reaches 0·12 mm. in length by 0·066 mm. thick; it crosses the ventral excretory canal (Fig. 3). There are about 50 to 60 rounded testes of which 15 to 20 are poral in position and the rest aporal; they are from 0·03 to 0·039 mm. in diameter; the area between these testicular groups is occupied by the somewhat centrally placed female glands, which in the material available, have not yet attained maturity. The oldest segment are 0·516 mm. broad by 0·168 mm. long to 0·6 mm. broad by 0·216 mm. long.

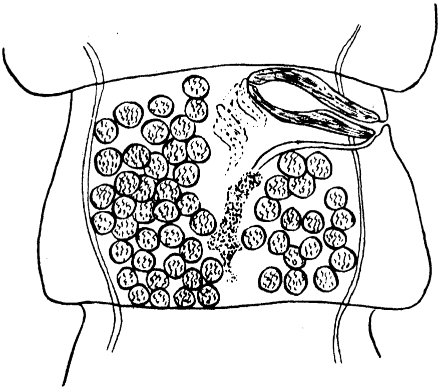


Fig. 3.—*Raillietina* (*S.L.*) *thryonomysi* sp. nov. Segment showing male genitalia.

Affinities.—The fairly large rostellar hooks, the collar of rostellar spines, the unarmed suckers and unilateral genital pores suggest that these parasites belong to the genus *Houttuynia* Fuhrmann, 1920. The absence of frayed endings to the handle of the hooks, the somewhat central position of the female glands, the presence of a cirrus sac which crosses the excretory canal and because of the lack of ripe segments showing the nature of the egg capsules has caused the writer to refer his material to the genus *Raillietina* Fuhrmann 1920 (S.L).

Among the mammalian forms of Davaineinae there is no species which combines the charcters enumerated above, and although the material is immature the writer thinks that the characters shown are sufficient to warrant the creation of a new species for its reception.

Specific diagnosis.—Davaineinae having a fairly large head, unarmed suckers, about 100 rostellar hooks arranged in two circles, anterior hooks 0·04 and posterior hooks 0·036 mm. long. Neck present, genital pores unilateral; cirrus sac crosses the ventral excretory canal; about 50 to 60 testes arranged in an aporal group and a poral group of 15 to 20 testes; between them the centrally placed female glands.

Host: *Thryonomys swinderianus variegatus* Ptrs.

Location: Small intestine.

Locality: Zululand, Natal.

Types in the Onderstepoort Helminthological Collection.

Idiogenes kori sp. nov.

About 50 specimens of this species were collected from the small intestine of a Giant Bustard; the acephalus strobilae vary in length from 7 mm. to 60 mm. with a maximum thickness of 0·72 mm. towards their posterior extremity. The strobilae are thin and delicate and in most cases are semitransparent. The anterior segments, of which at least eight are involved, are modified to form a pseudoscolex (Fig. 4); in these the segments are tent-shaped with their posterior margin standing away in an angle from the axis of the strobila and having this margin deeply indented in its middorsal and midventral lines; these indentations are less marked towards the posterior end of the pseudoscolex. In two strobilae, which were composed of 120 an 170 strobilae each, the genital organs appeared in the following segments respectively:—first genital primordia in 50th and 72nd segment; 1st ovarian primordia in 85th and 100th segment; mature segments in 98th to 104th and 130th and 137th segments; 1st appearance of paruterine organs in 112th and 146th segments and the last few segments of each strobila appeared to have fully formed paruterine organs, these organs reaching a length of 0·3 mm. by 0·19 mm. wide; no eggs however, had, entered these organs.

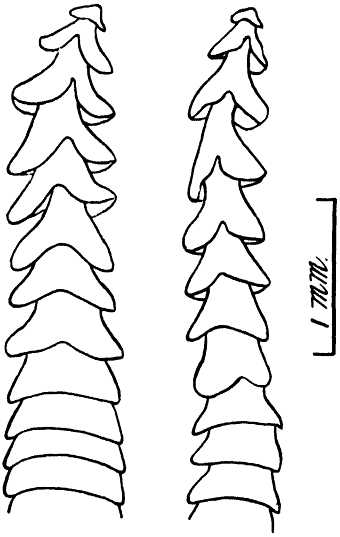


Fig. 4.—*Idiogenes kori* sp. nov. Pseudoscolices.

The genital pores are non-protuberant and are situated at the junction of the first and second body thirds. The cirrus sac is large, long and club-shaped (Fig. 5) and generally it extends obliguely inwards and forwards to reach the midline of the anterior margin of the segment; its wall consists of only a thin muscular sheath,

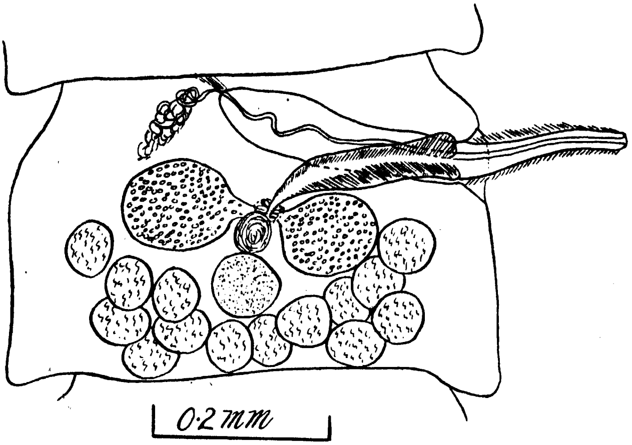


Fig. 5.—*Idiogenes kori* sp. nov. Mature segment.

and its length varies from 0·27 to 0·3 mm. by 0·07 to 0·078 mm. in diameter. A bunch of retractor muscles are attached to its anterior apex. The cirrus is massive and when fully exerted it reaches a length of 0·198 mm. and its base has a thickness of 0·039 mm. it is densely covered with prominent needle-like spines directed inwards; the spines being from 0·015 to 0·018 mm. long. When the cirrus is retracted the vas deferens is thrown into loops inside the cirrus sac, but when the cirrus is exerted the vas deferens is more or less straight; a vesicula seminalis interna appears to be absent. The testes, of which there are from 12 to 15 in each segment, are all situated behind the ovary; they are arranged in two layers in the medulla, and are rounded with a diameter of 0·06 mm. The vagina opens to the exterior immediately ventral of the cirrus pouch; it is practically straight with only its inner tip bent backwards; it is massive, having a maximum thickness of 0·045 mm. near its external orifice and being about 0·03 mm. thick at its proximal end; the whole length of its lumen is lined by prominent inwardly directed spines. The two rounded ovarian lobes are placed one on either side of the midline in about the centre of the segment, the aporal lobe is generally slightly larger than the poral; their outline is generally smooth, but in some cases their edge appears slightly crenated; their diameters are about 0·09 and 0·11 mm .for the poral and aporal lobes respectively. A rounded and centrally placed receptaculum seminis is present. The vitelline gland lies immediately behind the receptaculum seminis; it is rounded to slightly oval, and its diameter is only slightly greater than that of the testes. The uterus first makes its appearance as a transverse tube behind the ovary; it rapidly becomes U-shaped but this shape is not apparent in older segments, because with the development of the paruterine organ, this organ presses on the anterior face of the uterus causing it to appear to consist of two isolated bags, one on either side of the paruterine organ; in no segment were there any eggs seen inside the paruterine organ. As the oldest segments available contained no mature eggs, it would appear as if they only ripen after detachment of the segments and also only then entered the paruterine organ.

Affinities.—Three species of this genus have so far been described from otidiform birds; these are *I. grandiporus* Cholodkovsky, 1905, *I. nana* Fuhrmann, 1925 and *I. otidis* Krabbe, 1867. The former species differs from the writer's in that it normally carries a scolex in the adult, the number of testes is greater (30) and the genital ducts open into a large and conspicuous cloaca. Fuhrmann's species differs in being much smaller (less than 10 mm. long), the pseudoscolex is formed of the first five segments, the testes are fewer (9-11) and the vagina is much longer, thinner and coiled. The above described species has its closeset relative in *I. otidis*, which species differs from it, however, by its thinner and coiled vagina which from the figures available, is also unarmed; in that the pseudoscolex is formed of the first four segments and in that the cirrus sac is much shorter in length (0·15 mm.).

Specific diagnosis.—Idiogeninae reaching a length of 60 mm.; scolex absent in adult and replaced by a pseudoscolex formed by at least the anterior eight segments. Genital pores unilateral; cirrus large, up to 0·3 mm. long; cirrus massive and densely covered by large spines; 12 to 15 rounded testes in posterior half of segment. Vagina massive and straight, its lumen lined by strong spines Ovarian lobes rounded, centrally placed on either side of midline. Uterus an inverted U, but pressure of parutenine organ on its anterior face causes it later to appear as two bags lateral of this organ.

Host: Choriotis kori (Burch.).

Location: Small intestine.

Locality: Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

Scolex of *Idiogenes kori*.

The writer is also tentatively referring to the above species six small strobilae carrying scolices, which were also present among the materials of the above described species. The longest strobila (Fig. 6B) is 1·8 mm. long, carries a scolex, 17 segments and a caudal appendage nearly half the length of the entire strobila; the other five strobilae are similar, only smaller and have less segments. In the figured strobila it appears as if the 3rd to 8th segments are beginning to assume the shape of the segments of the pseudoscolex, and if this is the case then either the 1st and 2nd segments, which in this strobila are not yet fully dilineated, are shed with the scolex, or else they only assume the characteristic shape of those of the pseudoscolex at a later period.

The scolex, which is very similar to that described for the next species, is roughly acorn-shaped, the acorn being represented by the large rostellum which is separated from the scolex proper by a constriction. The rostellum has a diameter of 0·174 to 0·21 mm. and carries a double crown of 44 to 50 hammer-shaped hooks (Fig. 6B) of which those of the anterior row are 0·046 to 0·048 mm. long and those of the posterior row 0·036 mm. long. The axis of the blade of the longer hooks forms an obtuse angle with the axis of the handle, whereas in the smaller hooks these axes form roughly a right angle. The whole rostellum, posterior of its hooks, is covered by innumerable minute spines. The scolex has a diameter across the suckers of 0·24 to 0·3 mm. and the four somewhat spherical suckers are unarmed and protuberant and have a diameter of 0·09 to 0·102 mm. The neck varies in length from 0·3 to 0·36 mm. and has a breath of 0·102 to 0·19 mm.

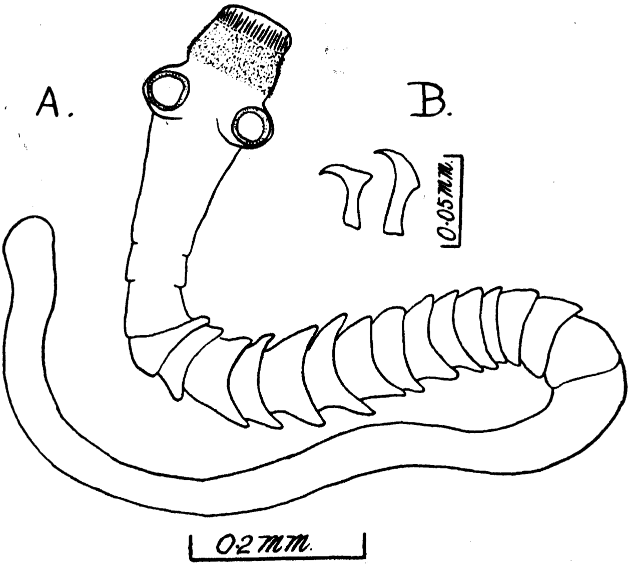


Fig. 6.—*Idiogenes kori* sp. nov. A = young strobila with scolex; B = hooks.

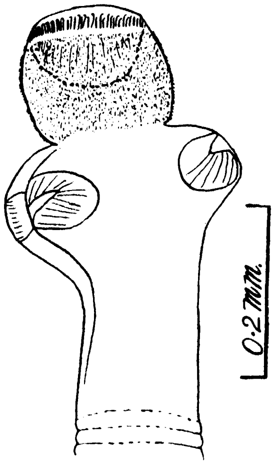
The shape of the scolex, the presence of hammer-shaped rostellar hooks, and collar of minute spines round the rostellum, the protuberant suckers, presence of a caudal appendage, and differentiation of the anterior segments, all these characters are very similar to those of the larval stages of *I. nana* figured by Fuhrmann (1925); also the scolex is very similar to that described for the next species and that figured by Cholodokovsky (1905) for *I. grandiporus.* In consequence of these similarities the writer is fairly confident that these small strobilae are very young stages of the species described above; this view is further supported by the fact that all the other cestode species harboured by this host had their scolices, so that these scolices had either to belong to the species described above or belong to a different species of cestode with which this host had only recently become infected.

Should further material prove that the view expressed above is correct, then the size and shape of the rostellar hooks would definitely show that this species is different to all the hitherto described species of this genus.

Idiogenes kolbei sp. nov.

From the same host which harboured the preceding species about 60 specimens of the above species were recovered; on examination it was noted that about 20 of these still carried theiir heads, but most of them had partially or completely lost their hooks; those without heads showed no signs of pseudoscolex and a cursory examination easily showed that their scolices had been separated and lost during collection.

The strobilae vary considerably in length, the longest reaching a length of 32 mm. with a maximum breadth of 0·54 mm.; they taper considerably towards their anterior ends and, in those specimens which are well extended, the strobilae are semi-transparent.



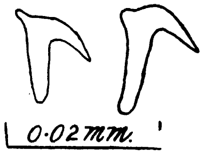


Fig. 8.—*Idiogenes kolbei* sp. nov. Hooks.

Fig. 7.—*Idiogenes kolbei* sp nov. Scolex.

The head (Fig. 7) is very similar in shape to that described for the preceding species; across the suckers its breadth varies from 0·22 to 0·27 mm.; the rostellum, when fully exerted is prominent, 0·12 to 0·16 mm. thick and carries a double crown of typical hammer-shaped hooks (Fig. 8); these hooks appear to vary in number from 120 to 140 and the larger ones measure 0·015 to 0·016 mm. in length and the smaller ones 0·012 to 0·013 mm. The suckers are relatively small, unarmed and rounded and have a diameter of 0·06 to 0·078. As in the preceding species the base of the rostellum is covered by innumerable minute spines. A short neck is present, from 0·15 to 0·21 mm. long by 0·114 to 0·132 mm. broad.

The longest strobila (32 mm.) consisted of 153 segments, but the most segments (183) were encountered in a strobila 31 mm. long. In two strobilae 31 and 24 mm. long with 176 and 150 segments each the organs appeared in the following segments respectively: first appearance of genital primordium 58th and 70th segment; first appearance of ovary 100th and 92nd segment; mature segments 125 to 133 and 112 to 121; first appearance of paruterine organ 141st and 132nd segment; mature eggs in 171st to 176th segments and 146th to 150th segments. No strobila carried mature segments in which the eggs had entered the paruterine organ.

The unilateral genital pores are situated in the anterior half of the segment, generally at about the junction of the first and second thirds; as a rule they are very prominent (Fig. 9A). The cirrus sac is large and extends obliquely forwards to about the middle of the anterior margin of the segment; it is weakly muscular, 0·19 to 0·21 mm. long by 0·066 mm. broad. The cirrus, which when extended has a thickness of 0·024 mm., is densely covered with small spines 0·006 mm. long; the vas deferens forms a few coils inside the cirrus and on emerging from it is thrown into numerous closely packed loops. A vesicula seminalis interna is absent. The testes are large and round and become mature before the ovary; they number from six to eight, have a diameter of about 0·06 mm. and are situated in the posterior half of the segment behind the ovary and between the excretory canals. The vagina opens immediately ventral of the opening of the cirrus sac; it is more or less straight, not forming any loops and its terminal third has its lumen widened to 0·024 mm. and is lined by small spines. The ovary is more or less centrally placed in the anterior half of the segment, and consists of two rounded to oval wings up to 0·12 mm. long by 0·06 mm. across; a small shell gland is situated at the junction of the two ovarian

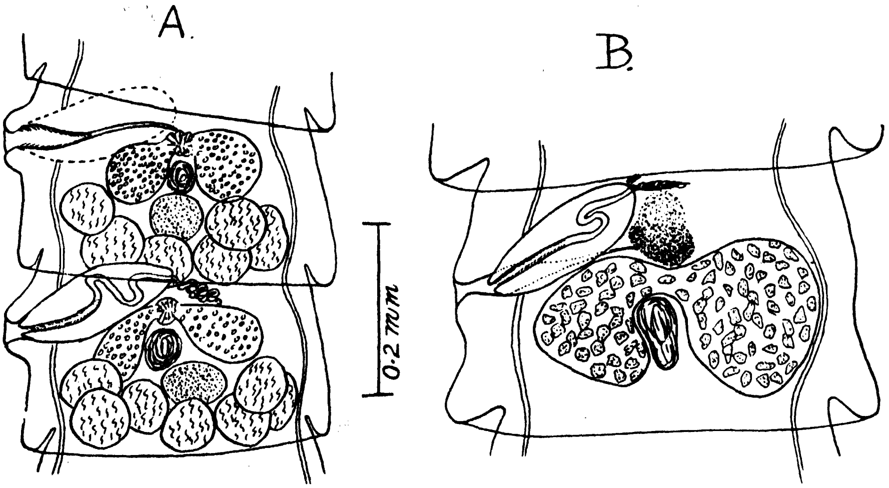


Fig. 9.—*Idiogenes kolbei* sp. nov. A = mature segments (the male duct has been omitted in the anterior and the female duct has been omitted in the posterior segment); B = nearly ripe segment.

lobes. The rounded receptaculum seminis is situated between the ovarian wings and has a diameter of about 0·045 mm. The yolk gland is generally oval, 0·066 mm. by 0·042 mm. and is generally centrally placed behind the receptaculum seminis; however, this latter organ may displace it slightly to the right or to the left of the midline. The uterus arises as an inverted U-shaped structure in the area occupied by the ovary; as it enlarges it fills up most of the space between the excretory canals and the cirrus sac leaving the space between its limbs free where the receptaculum seminis is found to persist (Fig. 9B). Soon after the paruterine organ makes its appearance as a darkly staining cell mass in the middle of the anterior half of the segment; it grows backwards and compressing the anterior and posterior walls of the uterus on to the receptaculum seminis causes the uterus to loose its U-shape and assume the appearance of two isolated lateral bags. In the hindmost segments the eggs appear to be mature but no eggs have entered the paruterine organ; it would thus appear that their entrance only takes place after the end segments have been shed. The eggs are thin-walled and round, and have a diameter of 0·038 to 0·042 mm. and the hexacanth hooks are 0·018 mm. long.

Affinities.—This species is easily differentiated from the preceding species in that a pseudoscolex is not formed, a scolex being normally present in the adult; the testes are fewer; the cirrus and vagina are not so massive and their spines are considerably smaller. The absence of a pseudoscolex distinguishes this species from *I. otidis* Krabbe, 1867 and *I. nana* Fuhrmann, 1925, and the smaller and greater number of rostellar hooks, fewer testes and absence of large genital atrium differentiates it from *I. grandiporus* Cholodk, 1905.

Specific diagnosis.—Idiogeninae reaching 32 mm. in length by 0·54 mm. broad. Scolex normally present in the adult; pseudoscolex absent. 120 to 140 rostellar hooks of typical shape and about 0·013 and 0·016 mm. long. Rostellum covered by innumerable small spines. Genital pores unilateral. Cirrus sac large, reaching 0·21 mm. in length; cirrus covered with small spines; vagina straight, its end third has a widened lumen lined with small spines. Testes six to eight in posterior half of segment. Ovary two-lobed; uterus U-shaped. Eggs rounded, and do not enter paruterine organ while segment is still attached to strobila.

Host: Choriotis kori (Buch.).

Location: Small intestine.

Locality: Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

The writer has much pleasure in naming this species in honour of Mr. F. F. Kolbe, B.Sc., Officer in Charge of the Zoological Survey in the field, who was personally responsible for collecting all the material from this host.

Schistometra connoideis (Bloch, 1782).

About a dozen full-grown specimens were recovered from a Giant Bustard shot in the Northern Transvaal. In addition about 50 immature specimens were present ranging in length from about 10 mm. to 60 mm. with a maximum breadth of 4 mm. The longest mature specimens were from 150 to 175 mm. long with a maximum breadth of 9 mm.

This species was first recorded from this host by Beddard (1912) as *Otiditaenia eupodotidis* gen. and sp. nov. The writer fully agree with Skrjabin (1914) that this species is cospecific with Bloch's (1782) species (syn. *S. togata* Cholodk, 1912) as an examination of the writer's material shows. The suckers each carry two muscular appendages, each about 0·06 mm. long. The rostellum carries about 500 small and typical hammer-shaped hooks, in two regular circles; those of the anterior row are 0·01 mm. long and those of the posterior row ·0085 mm. long. The base of the rostellum is densely covered with minute spines. The ovary is small and decidedly poral in position, being located just internal to the poral ventral excretory canal and anterior of the testes. The receptaculum seminis is fusiform and increases in size posteriorwards; in mature segments it is about 0·18 mm. long by 0·06 mm. thick and in the hindmost segments 0·48 mm. long by 0·1 mm. thick. The uterus arises as a transverse canal immediately anterior of the testes and extends across the whole segment between the excretory canals; near the excretory canals it becomes enlarged; its cavity in ripe segments is traversed by trabeculae which divide it into cavities extending antero-posteriorly; these cavities are again sub-divided to form irregular cavities each containing a single egg. In sections of the last few segments of three worms (Fig. 10) it was noted that the eggs had entered the paruterine organs in the last two segments of each worm. When entering this organ three of four flask-shaped paruterine capsules are formed for the reception of a considerable

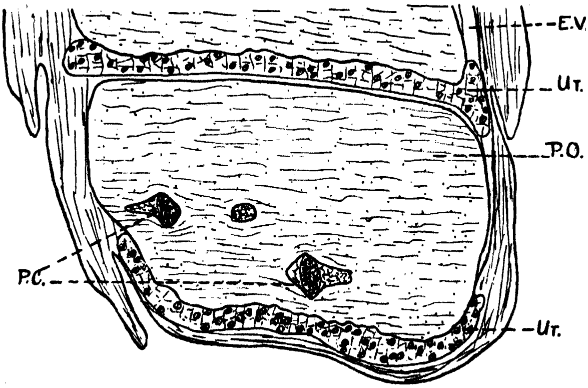


Fig. 10.—*Schistometra conoideis.* Horizontal section of hindmost segment. E.V. = excretory vessel; P.C. = paruterine capsules; P.O. = paruterine orgen; U.T. = uterus.

number of eggs. These capsules arise from the posterior face of the paruterine organ and become connected with the uterus; after having received their eggs they become severed from the uterus and pass forwards to lie separately in the posterior half of the paruterine organ. The capsules have a fibrous wall, and consist of a " body " and " neck ", the latter generally kinked (Fig. 11A); the whole capsule has an overall length of about 0·42 mm. of which 0·2 mm. represents the neck and the remainder forms the body measuring about 0·22 by 0·26 mm. The " body " is filled by a considerable number (roughly about 60) of oval and thin-walled eggs having an average size of 0·042 by 0·025 mm. and the hexacanth hooks measure 0·014 mm. (Fig. 11B). The whole mass of eggs is enclosed in a thin membrane separating them from the " body " of the capsule. From the literature available the writer has not been able to find any definite reference to these paruterine capsules. Beddard (1914) is unfortunately not clear on this point, in that when discussing the ultimate fate of the uterus he states (p. 218) " the eggs are at the very end grouped into many more or less isolated but not well marked causples "; whether he refers to the uterine or paruterine capsules is not clear, although on the previous page he states that there is a " tendency for the fibrous parenchyma to enwrap the bundles of eggs " which would suggest that these egg bundles were lodged in the paruterine organ. His figure of a section of a ripe segment (Fig. 30) shows groups of eggs in the paruterine organ but these do not appear to be enclosed by fibrous capsules.

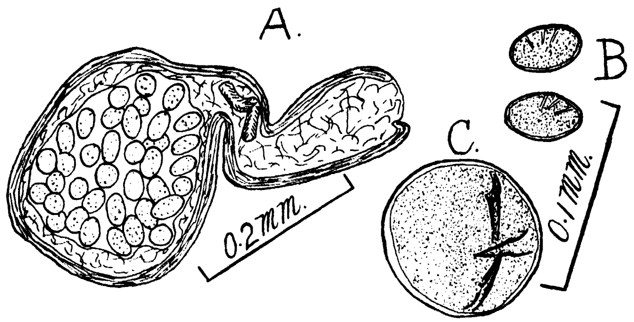


Fig. 11.—*Schistometra conoideis.* A = section of paruterine capsule with eggs; B = eggs from paruterine capsule; C = eggs from uterus of same segment (B and C are equally enlarged).

A remarkable feature of the eggs in the paruterine capsules is that they are very much smaller than the apparently ripe eggs still present in the uterus and the hexacanth hooks are also much smaller; eggs in the uterus of the same segment containing paruterine capsules were globular, had a thin and smooth shell, measured from 0·066 to 0·084 mm. in diameter and their hexacanth hooks were from 0·037 to 0·039 mm. long (Fig. 11c). How this change takes place, especilly in the size of the hexacanth hooks, the writer is unfortunately not able to say, as he did not observe any capsules into which the eggs were in the process of entering. The possibility of two types of eggs being present in the uterus is also excluded as a careful search of a number of sections through different uteri only showed the presence of round large eggs.

A curious feature of the paruterine capsules is that the neck contains a short coiled duct, about 0·015 mm. in diameter and having its internal surface lined by short spines.

The arrangement of the testes is typical for the genus consisting of a vertical sheet at the hind end of the segment; there are from 25 to 34 testes in a transverse row and there are five or six of these rows one above the other. A vesicula seminalis is absent, but the vas deferens is much coiled after emerging from the cirrus sac which reaches but does cross the excretory canal; this latter organ is about 0·24 mm. long by 0·09 to 0·01 mm. thick (Skrjabin says 0·6 mm. long by 0·2 to 0·25 mm. broad). In all the transverse sections, obtained from different parts of two adult worms, no trace of a dorsal excretory canal was seen. The genital ducts passed dorsal of the ventral excretory canal and nerve.

Host: *Choriotis kori* (Buuch.)

Location: Small intestine.

Locality: Northern Transvaal.

Schistometra sp.

The writer is tentatively referring to this genus two very immature strobilae obtained in association with the preceding species. The strobilae are respectively 10 and 15 mm. long. The head is very similar to that of *S. korhaani* Ortlepp 1938; its rostellum is covered by minute spines and also a double circle of about 500 hooks arranged in a zig-zag and having a break at each lateral corner; the hooks, however, are longer than in this species being 0·021 and 0·018 mm. long for the anterior and posterior row respectively. Each sucker is provided with two muscular appendages.

Host: *Choriotis kori* (Buuch.)

Location: Small intestine.

Locality: Northern Transvaal.

Hymenolepis cormoranti sp. nov.

About a dozen specimens of this delicate cestode were collected from two or three Reed Cormorants. Only two worms still had their solices present but a few additional detached scolices were recovered from scrapings of the intestine.

The total length reaches 150 mm. with a maximum thickness of 0·6 mm. at the posterior end; the strobila is thin, especially in its anterior half which has the appearance of a thread of white cotton.

The head is small and only reaches 0·138 mm. across; it is somewhat spindle-shaped. In all cases the rostellum is retracted and its sac extended backwards for about 0·13 mm. posterior of the level of the suckers; it carries a single row of ten small hooks (Fig. 12A) 0·024 to 0·025 long, having a long handle and a blade only 0·006 mm. long. The suckers are slightly oval and small and measured 0·054 by 0·06 mm. across.

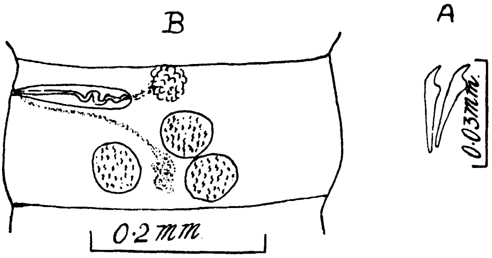


Fig. 12.—*Hymenolepis cormoranti* sp. noc. A = rostellar hook; B = segment with mature testes.

The genital pores are unilateral and situated in the anterior quarter of the segment's margin. The cirrus sac is elongate and tubular, crosses the excretory canal and reaches the poral testes and sometimes extends beyond it; it is about 0·15 mm. long by 0·03 mm. in thickness, but in older segments it may even reach a length of 0·17 mm.

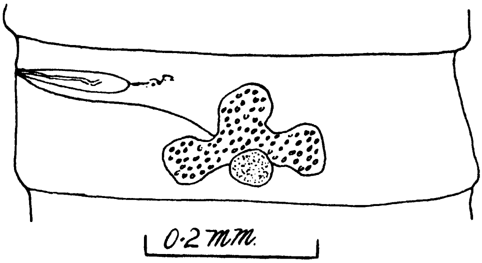


Fig. 13.—*Hymenolepis cormoranti* sp. nov. Segment with mature ovary.

The three testes, which are rounded and have a diameter of 0·045 to 0·06 mm., are arranged in the form of a triangle, one poral and two aporal (Fig. 12B); the anterior aporal testes it situated slightly more internal than that behind it. In segments, where the testes had attained maturity, the female glands are only represented by a darkly staining mass of cells; as the testes begin to disintegrate the ovary begins to assume a definite shape, and further back when the testes have completely disappeared the ovary and its associated glands become mature; in these segments (Fig. 13) the ovary is trilobed and measured from 0·17 to 0·2 mm. across by about 0·08 in length. The yolk gland lies immediately behind the ovary, it is rounded and has a diameter of about 0·05 mm.

Affinities.—Baer (1933) identified fragments of a cestode obtained from the same host as *H. phalacrocorax* (Woodland, 1929), although he noted that his material showed several differences from Woodland's description based on material obtained from an Indian Large Cormorant (*Phalocrocorax carbo*). Although Woodland found no hooks or rostellum on the one available scolex, it is not certain whether this species is normally unarmed as this scolex was much distorted. Baer's material unfortunately contained no scolex. Apart from the armed scolex the writer's specimens differ from both Woodland's and Baer's specimens in that the testes are not found external of the excretory vessels, in that the cirrus sac is a simple muscular tube and not the complicated structure described for Woodland's species, and in that the ovary is trilobed; from Baer's specimen it differs in that the cirrus sac is much longer and extends almost to the middle of the segment, the peculiar structure of the vagina was not noted, and the testes are much smaller.

The number, size and shape of the rostellar hooks easily distinguishes this species from the species *H. ficticia* (Meggitt, 1929), *H. magniuncinata* Meggitt, 1927, *H. parvicirrosa*, Meggitt, 1927, and *H. medici* (Stossich, 1890), from Pelicaniformes; *H. parviuncinata* Meggitt, 1927, also from this group of birds, has the same number of hooks (10) as in the writer's species, but they are much smaller (0·013 to 0·018 mm.) and their shape is also different.

Specific diagnosis.—Hymenolepididae attaining a length of 150 mm. or more by 0·06 mm. broad; scolex small, 0·138 mm. across; rostellum with 10 hooks 0·024 to 0·025 mm. long having a long handle and small blade. Genital pores unilateral. Three testes, one poral and two aporal, disappear before appearance of female glands; cirrus long, crosses excretory canal and reaches poral testes. Ovary trilobed, large; yolk gland round; uterus a transverse bag, extends across excretory canal to edge of segment.

Host: *Microcarbo africana africanoides* (A. Smith).

Location: Small intestine.

Locality: Pretoria district, Transvaal.

Types in the Onderstepoort Helminthological Collection.

Class **NEMATODA.**

Contracaecum carlislei sp. nov.

The Reed Cormorants from which this species was obtained were shot by Mr. Carlisle of this Institute and placed at the writer's disposal for section: the writer has much pleasure in naming this species after Mr. Carlisle in recognition of his services for having from time to time placed materials for section at his disposal.

The above species was represented by about two dozen specimens, representing fourth stage larvae, adolescent and mature worms. They were found firmly attached to the inner lining of the oesophagus and stomach.

The worms are creamy yellow in colour and attenuated towards both extremities; the mature worms reach a length of 10 mm. by 0·5 mm. broad for the males and 27 mm. by 1 mm. thick for the females. The three lips are separated from the body by a constriction and each is somewhat rectangular, being slightly longer than broad and each is bilobed anteriorly having the lateral corner of each lobe drawn out into a point (Fig. 14); in the males they reach a length of 0·075 mm. and in the females 0·84 mm.; their pulps are provided with two swollen processes which extend one into each of the anterior lobes of the lips. The dorsal lip carries two single papillae but the sub-ventral lips each carry a ventral double and a dorsal single papilla. The interlabia are large and curved, being almost as long as the lips. Dentiginous ridges are absent.

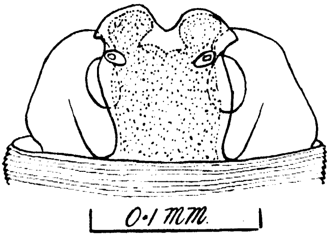


Fig. 14.—*Contracaecum carlislei* sp. nov. Dorsal lip.

The body is transversely striated, those striae just behind the lips being more prominent and giving this portion of the body a serrated appearance. The tail is short and conical in both sexes, being about 0·25 mm. long in the male and 0·24 to 0·264 mm. long in the female. Lateral alae are absent.

The oesophagus is 3·1 to 3·7 long and increase slightly in thickness posteriorly; in the male its anterior and posterior diameters are 0·084 and 0·12 mm. and in the female 0·108 and 0·192 mm. respectively; its ventral caecum is 0·78 mm. long in the male and 0·86 mm. long in the female. The intestinal caecum is long and extends to almost 0·5 mm. behind the level of the nerve ring, being 2·76 mm. long in male 2·25 mm. long in the female. The nerve ring is 0·48 to 0·52 from the anterior end and the two small cervical papillae are situated about 0·1 to 0·12 mm. behind the nerve ring.

The tail of the male carries no alae; there are 6 pairs of post cloacal papillae (Fig. 15); the first two pairs are longer and ventrolateral in position; they are situated close together and only a careful examination reveals the fact that they are two separate papillae and not a single doubled one; the remaining four pairs are small, and situated in the posterior half of the tail, two pairs being lateral and two pairs ventral. The spicules are equal 2·2 to 2·4 mm. long by 0·036 mm. broad at their proximal ends; they are alate in their posterior half. There are 30 or more pairs of precloacal papillae extending forwards on either side from the cloaca. A gubernaculum is absent.

The vulva is slightly protuberant and situated in the anterior body half; its position divides the body roughly into the ratio of 2 : 3; the vagina is about 1·2 mm. long by 0·15 mm. thick and its lumen has a diameter of 0·06 mm.; it may extend transversely across the body and then bend backwards in a right angle, or it may first pass obliquely forwards for about 0·5 mm. and then bend backwards.

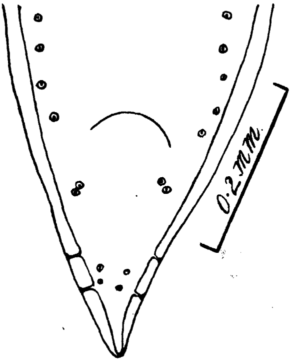


Fig. 15.—*Contracaecum carlislei* sp. nov. Ventral view of male tail.

The numerous eggs are rounded and relatively thin shelled; they are from 0·051 to 0·054 mm. in diameter and their shells are 0·003 to 0·004 mm. thick.

Affinities.—The nature of the labial papillae and the arrangement of the male postcloacal papillae shows that this species is closely related to *C. rodhaini* (Gedoelst, 1916) from *Plotus rufus;* the only difference being that in Gedoelst's species the subventral lips each carry only a double papilla and the first postcloacal papilla is double; however, more important differences are that in *C. rodhaini* the spicules are much longer (3·6 mm.), the vulva is more anterior in position (divides body into ratio of 3 : 7), the male tail is shorter (0·75 mm.), the female tail is longer (0·336 mm.), and the bases of the lips are more cut-in than in the writer's species.

The size and shape of the spicules and position of the vulva are very similar to those of *C. microcephalum* (Rud., 1809), but this species has two double papillae to each dorsal lip and a single double papilla to each sub-lateral lip; in addition the arrangement of the post-cloacal papillae are also different.

The size of the spicules distinguishes the writer's species from the recently described species *C. torquatum* Yamaguti, 1935, *C. milvi* Yamaguti, 1935 and *C. hagedashiae* Sandground, 1933.

Specific diagnosis.—Anasakinae reaching a length of 15 mm. for the males and 27 mm. for the females. Three lips somewhat rectangular and bilobed anteriorly; inter-labia large and curved; dorsal lip with two single papillae and subventral lips each with a double and a single papilla. Intestinal caecum long. Tail in both sexes about 0·25 mm. long. Spicules equal, alate, 2·2 to 2·4 mm. long; gubernaculum absent; 30 or more pairs of precloacal papillae and six pairs of postcloacal papillae of which the papillae of the 1st and 2nd pairs are close together but nor forming a double papillae. Vulva divides body into ratio of 2 : 3. Eggs round and thin-shelled.

Host: Microcarbo africana africanoides (A. Smith).

Habitat: Oesophagus and stomach.

Locality: Pretoria district, Transvaal.

Types in the Onderstepoort Helminthological Collection.

Porrocaecum spathulespiculum sp. nov.

The materials on which the following description is based consists of two males and one female, all mature, from one bird host (unidentified), and two males and two females (all mature) and two immature females from another bird host (unidentified). They are creamy white in colour and are attenuated towards both extremities in both sexes. The males are from 32 to 44 mm. long with a maximum thickness in their middle of 1·17 mm. and the females are 34 to 65 mm. long with a maximum thickness behind the vulva of 1·6 mm. The cuticle is transversely striated, and cervical and caudal alae are absent.

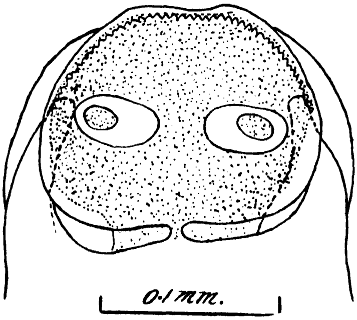


Fig. 16.—*Porrocaecum spathulespiculum* sp. nov. Dorsal lip.

The three lips are rounded and slightly indented anteriorly and are deeply cut in at their bases (Fig. 16); their length varies from 0·145 mm. to 0·16 mm.; the labial papillae are all simple, large and dome-shaped, two being present on the dorsal lip and one on each subventral lip. Each lip carries an internal dentigerous ridge whose denticles are largest towards the apex of each lip. The pulp of the dorsal shows no special peculiarities and simply follows the outline of the lip. The interlabia are large and prominent and about two-thirds the length of the lips. The oesophagus, including its ventriculus, is from one-tenth to one-twelfth of the total body length and increases slightly in thickness posteriorly, where it may reach a thickness of 0·45 mm.; its ventriculus is about 0·75 mm. long. The intestinal caecum is small and inconspicuous and only reaches 0·18 mm. in length. In a male 32 mm. long the nerve ring was 0·6 mm. from the anterior end, and the small cervical papillae were situated 0·24 mm. behind the nerve ring.

The vulva is non-protuberant and situated in the anterior body half. its position dividing the body into the ratio of 8 : 11. The vagina is relatively short and soon leads into a large egg reservoir passing posterior-wards; two uteri originate from its posterior end. The eggs are oval and have pitted shells; they are from 0·084 to 0·1 mm. long by 0·06 to 0·072 mm. in thickness; their shells are from 0·003 to 0·004 mm. thick. The tail is somewhat stumpy and is from 0·74 to 0·84 mm. long (Fig. 17).

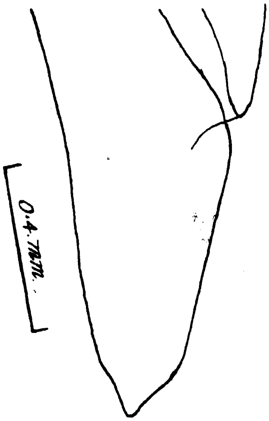


Fig. 17.—*Porrocaecum spathulespiculum* sp. nov. Female tail.

The caudal extremity of the male is pointed and the tail is from 0·33 to 0·43 mm. long (Fig. 18A); there are five pairs of postcloacal papillae, that immediately behind the cloaca being double; the remaining four pairs are equidistant from each other and placed further back. There are 12 to 14 pairs of precloacal papillae. The two spiclues (Fig. 18B) are equal, robust, slightly arched and alate, the alae extending from just behind the head to the tip and giving the spicule the shape of a flour scoop; they are from 0·78 to 0·792 mm. long by 0·098 mm. broad at their head. A gubernaculum is absent.

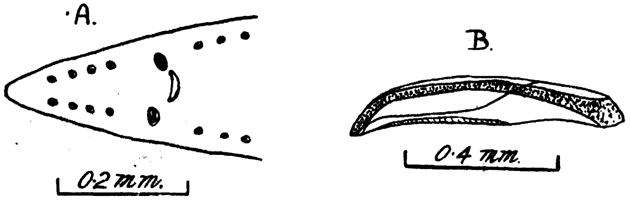


Fig. 18.—*Porrocaecum spathulespiculum* sp. nov. A = ventral view of male tail; B = side view of spicule.

Affinities.—The nearest relatives of the above described species appear to be *P. angusticolle* (Molin, 1860) and *P. depressum* (Zeder, 1800), both of which possess a pair of double papillae immediately behind the cloaca and four additional pairs of single papillae; the former species differs from the writer's in that its interlabia are small, its spicules are longer and non-alate and its intestinal caecum is much longer; Zeder's species differs from the writer's by the shape of the pulp of its dorsal lip, the connical tail appendage in the male and its small interlabia.

Specific diagnosis.—Anisakinae reaching a length of 44 mm. in the male and 65 mm. in the female. Lips somewhat rounded and deeply cut in at their bases; pulp of dorsal lip simple; dentigerous ridges present; two simple papillae and one simple papilla on the dorsal and subventral lips respectively; interlabia large. No cervical alae. Intestinal caecum very small. Vulva in anterior body half. Eggs oval and pitted. Five pairs postcloacal and 12 to 14 pairs precloacal papillae in male; first postcloacal papillae double. Spiclues, equal, alate and scoop-shaped. Gubernaculum absent.

Host: Bird (unidentified).

Location: Small intestine.

Locality: Transvaal.

Types in the Onderstepoort Heminthological Collection.

Subulura otidis sp. nov.

Numerous specimens were recovered from a single Giant Bustard. Unfortunately all the females were still immature, and consequently the measurements given below must be taken as applying only to young individuals which have not attained maturity.

The body is whitish in colour and is strongly arched dorsalwards at the anterior end. The body is thickest at the level of the junction of the oesophagus and intestine; from this point the body becomes thinner towards both extremities, this attenuation being less marked towards the anterior than towards the posterior end. In the females the body becomes very much attenuated posteriorly to end in a long and finely pointed tail; in the male, as far as the cloaca, the attenuation is not so marked, but in the tail region the body becomes considerably thinner to terminate in an elongate tail spike. The body cuticle shows fine annulations and also carries two long lateral alae which extend down the body for about two-fifths of its length; they are from 0·025 to 0·036 mm. high and are vertically striated. The mouth is simple and leads into a buccal capsule having thickened cuticular walls (Fig. 19); it has a diameter of 0·021 to 0·024 mm. at its anterior end and 0·040 to 0·045 mm. at its posterior end, and its average depth is 0·03 mm. Protruding into its base are three prominent teeth lodged on the apices of the three oesophageal segments. There are two wart-like lateral papillae and four small and dome-shaped submedian papillae round the mouth. The oesophagus is typical in shape and is from 1·27 to 1·34 mm. long in the males and 1·44 to 1·54 mm. in the females.

The length of the females is 9·0 to 11·6 mm. with a maximum thickness of 0·264 mm. The tail (Fig. 20A) is long, slender and pointed and averages 1·0 mm. in length. The vulva is inconspicuous and situated in the anterior body half, dividing the body roughly into the ratio of 4:5. The ovejector (Fig. 20B) is very short, its total length being from 0·516 to 0·544 mm.; its vestibule is bent sharply forwards and is from 0·17 to 0·17 mm. long; the sphincter measures only 0·7 mm. in length and the trompe is from 0·26 to 0·3 mm. long. As the material is still immature no eggs are present.

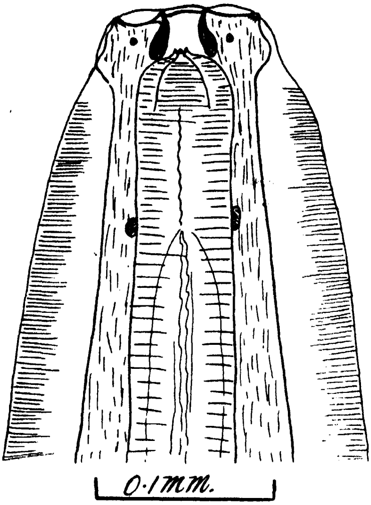


Fig. 19.—*Subulura otidis* sp. nov. Anterior extremity, dorsal view.

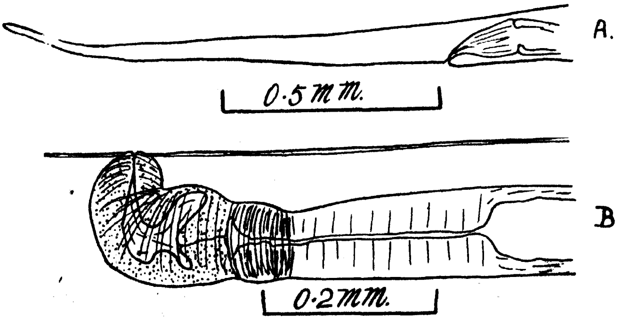


Fig. 20.—*Subulura otidis* sp. nov. A = female tail; B = ovijector.

The males are from 7·7 to 9 mm. long and have a maximum thickness of 0·24 mm. The caudal extremity (Fig. 21) is hooked ventralwards and is provided with very narrow alae. There are 10 pairs of caudal papillae of which two are precloacal, three circumcloacal and five post cloacal on the anterior half of the tail; a pair of small caudal pores is situated just anterior of the last pair of caudal papillae. The tail is relatively long, being from 0·36 to 0·4 mm. in length, and carries a long caudal appendage. The spicules are equal and similar, measuring 0·676 to 0·7 mm. in length; they are alate and their sharp tips are hooked ventralwards. The triangular gubernaculum is from 0·06 to 0·066 in length. The sucker is not very muscular and is situated 0·3 to 0·36 mm. anterior of the cloacal aperture.

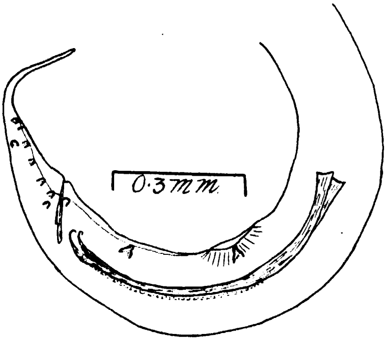


Fig. 21.—*Subulura otidis* sp. nov. Male tail, lateral view.

Affinities.—Of the species *Subulura* only the following have no lips and equal spicules less than 0·5 mm. long namely, *S. poculum* (v. Linstow, 1909), *S. forcipata* (Rud, 1819), and *S. noctuae* (Seurat, 1914). These species differ from the writer's in that the cervical alae are practically limited to the oesophageal region of the body. The long cervical alae appear to ally the writer's species to *S. leprincei* (Gendre, 1909) but this species differs in that it is stated to possess 13 pairs of caudal papillae, the spicules are 1 to 1·4 mm. long and the ovejector is very long.

Three species of this genus have, as far as the writer has been able to ascertain, been recorded from otididiform birds, namely *S. halli* Barreto, 1918 (=*S. forcipata* (Rud.) of Seurat, 1914), from *Otis tetrax*, *S. rima* (v. Linstow, 1906) from *Otis houbara* (=*Houbara undulata* and *S. suctoria* (Molin, 1860) from *Houbara macquenii*. The first and last of these differ from the above described species in that the spicules exceed 1·0 mm. in length. *S. rima* differs in that it is reported to have six oesophageal teeth, nine pairs of caudal papillae in the male and the spicules are unequal in length.

Specific diagnosis.—Subulurinae having a whitish colour in the preserved state and reaching a length of at least 9 mm. for the males and 11·6 mm. for the females. Lips absent. Cuticularized buccal capsule present. Cervical alae long extending to posterior quarter of body. Vulva in front of middle. Ovejector very short. Female tail long and pointed. Caudal alae of male very narrow. Ten pairs of caudal papillae. Spicules equal, similar and alate reaching 0·7 mm. in length.

Host: Choriotis kori (Burch.).

Location: Caeca.

Locality: Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

Cooperiodes hepaticae sp. nov.

This species is represented by 13 males and 9 females all collected from small nodules on the terminal portions of the bile ducts of an Impala. They are slender worms having a reddish colour. All the worms appear to be fully grown notwithstanding the fact that no female contained any eggs. The peculiar habitat of these worms is probably the reason for this, and it would suggest that this is not their normal location.

The head (Fig. 22A) is slightly swollen and is provided with four inconspicuous lips, carrying four submedian and two lateral papillae which show up as bright refringent dots when the worms are cleaned in creosote; the head of the female is up to 0·055 mm. broad and that of the male up to 0·05 mm. and the length of the cephalic swelling reaches 0·026 mm. in the female and 0·025 mm. in the male; in the two sexes the diameter of the neck, immediately behind the cephalic swelling, is 0·052 mm. and 0·043 mm. respectively; the neck is encircled by 8 to 10 coarse annulations, and behind these the cuticle shows the normal fine striations. Extending from behind the neck almost to the posterior end of the body the cuticle carries 10 longitudinal alae showing very regular and distinct vertical markings; these alae are from 0·01 to 0·017 mm. high, and the two alae immediately lateral to the vulva may in this region reach a height of 0·028 mm. Cervical papillae are absent.

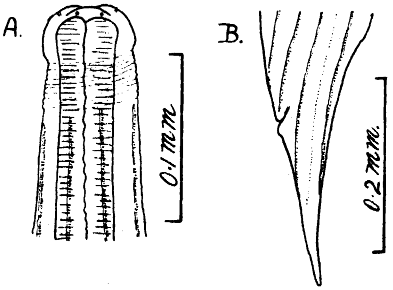


Fig. 22.—*Cooperiodes hepaticae* sp. nov. A = anterior extremity; B = female tail.

Female.—The females are from 15 to 17 mm. long with a maximum thickness of 0·29 mm. just in front of the vulva in the longest female. The tail (Fig. 22B) is pointed and is from 0·16 to 0·175 mm. long. The club-shaped oesophagus is from 0·72 to 0·754 mm. long and has a maximum thickness of 0·064 mm. at its posterior end; the excretory pore is situated 0·523 to 0·551 mm. from the anterior end and the nerve ring is situated about 0·075 mm. in front of the excretory pore. The vulva is not prominent and is found in the posterior body sixth; it is from 0·25 to 0·26 mm. from the tail tip and is bordered laterally by the enlarged cuticular alae. The ovejectors are of the usual trichostrongylid type and their combined length in the largest female is 0·638 mm. Eggs are entirely absent.

Males.—The males are from 11 to 13 mm. long and have a maximum thickness of 0·215 just anterior to the bursa. The oesophagus is 0·625 to 0·64 mm. long, and the excretory pore is 0·425 mm. from the anterior end; the nerve ring is situated 0·7 mm. anterior of the excretory pore. The caudal bursa consists of two large lateral lobes with numerous small tubercles on their ventral surfaces, and a small median lobe; the entire length of the lateral bursal lobes reach 0·32 mm. and the dorsal lobe 0·145 mm. The arrangement and proportions of the rays are best seen in the accompanying figures (Fig. 23A and B). The spicules are dark brown and equal in length and each is provided with a dorsal spur 0·05 mm. long (Fig. 23C); the body of the spicules end in a blunt tip tilted ventralwards; a thin membrane is supported by the spur and also by the spicule itself. The spicules are from 0·172 to 0·203 mm. long and have a maximum thickness at their anterior end just behind the head of 0·02 mm. A gubernaculum and prebursal papillae are absent.

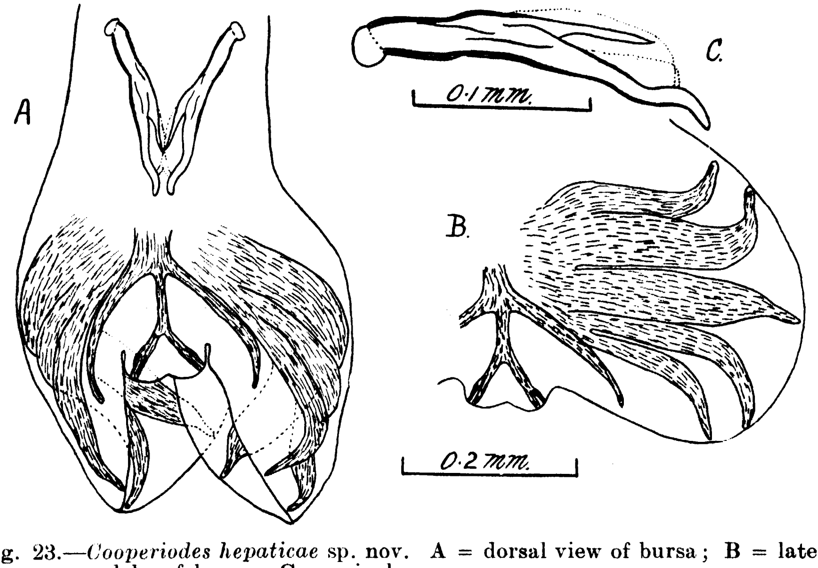


Fig. 23.—*Cooperiodes hepaticae* sp. nov. A = dorsal view of bursa; B = lateral lobe of bursa; C = spicule.

Affinities.—This species is closely related to *Cooperiodes hamiltoni* (Mönnig, 1933) from the same host as shown by the similar spicules and arrangement of the lateral and ventral rays; however, it, can be easily distinguished from this species by its larger size and by its much shorter dorsal ray and consequent smaller dorsal bursal lobe.

Specific diagnosis.—Trichostrongilidae reaching a length of 17 mm. in the female and 13 mm. in the male. Head swollen, about 0·05 mm. thick. Body carries 10 longitudinal alae extending from the neck to the anal region, each ala ornamented by numerous coarse vertical markings. Vulva in the posterior body sixth. Tail pointed. Bursa with two large lateral lobes and much smaller dorsal lobe;

bursal rays as in *C. hamiltoni* (Mönnig) but dorsal ray is much smaller. Spicules equal and each carries a dorsal spur; they are alate and from 0·172 to 0·203 mm. long. Gubernaculum absent.

Host: *Aepyceros melampus melampus* (Licht.)

Location: Small nodules on terminal portion of bile ducts.

Locality: Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

Trichonema (*Cylicocyclus*) *gyalocephaloides* sp. nov.

About 45 specimens, of which four were males, were present in a collection of helminths recovered from a zebra. Unfortunately every specimen, except one male, had burst, and consequently the lengths given below are smaller than the parasites would have been had they been entire. The worms have a brownish red colour and the intestine shows through the body wall as a dark line; the females are 13 to 15 mm. long, are attenuated towards both ends and have a maximum thickness of 0·6 to 0·72 mm. at about the middle of the body. The entire male is 14 mm. long by 0·53 mm. in its middle.

The anterior extremity is slightly swollen and resemble in shape that of *Gyalocephalus capitatus* (Fig. 24A). The mouth collar is rounded and flattened and measures 0·23 to 0·245 mm. across by about 0·03 mm. high; externally it carries two wart-like lateral papillae and four submedian papillae with nipple-like ends. The buccal capsule is large and somewhat barrel-shaped with a conspicuous hoop round its posterior edge; its anterior opening is slightly

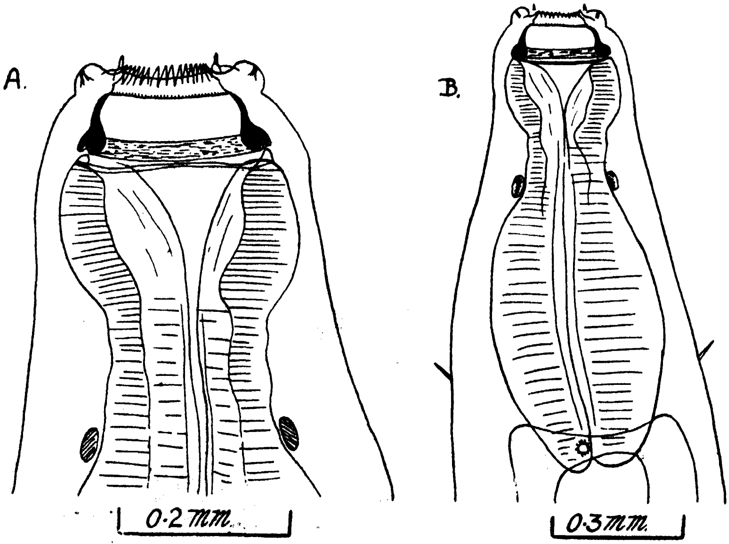


Fig. 24.—*Trichonema* (*Cylicocyclus*) *gyalocephaloides* sp. nov. A and B = anterior extremities.

less than its posterior and its maximum diameter is just anterior of its middle; it is from 0·075 to 0·09 mm. deep and its maximum diameter is from 0·186 to 0·204 mm. The external leaf crown originates about midway up the mouth collar and consists of 44 pointed elements about 0·03 mm. long; they project slightly above the anterior margin of the mouth collar. The internal leaf crown consists of numerous (Ca. 200) small elements, about 0·01 mm. long, inserted along the anterior edge of the buccal capsule. The excretory pore is situated at the level of the junction of the oesophagus and intestine, and the spike-like cervical papillae are inserted 0·12 mm. anterior of this level. The oesophagus is strikingly club-shaped (Fig. 24B) in that its anterior or head end is much enlarged to accomodate a cup-shaped oesophageal funnel; this portion measures 0·27 to 0·3 mm. across by about 0·2 mm. long, and its cavity is from 0·12 to 0·15 mm. deep by 0·18 to 0·19 mm. broad; its internal surface is lined by cuticle forming longitudinal folds. The following portion or oesophageal neck is considerably narrower and measures from 0·14 to 0·18 mm. in thickness; the nerve ring encircles it at its junction with the following much thicker oesophageal portion; this latter is from 0·33 to 0·42 mm. thick. The whole organ is from 1·03 to 1·14 mm. long.

The caudal bursa consists of a long median and two large lateral lobes (Fig. 25). The former is supported by the dorsal ray which is split almost from its base, and each limb carries three main branches which extend to the bursal edge. The externo-dorsal takes its origin from the dorsal ray just after its division into its two main limbs; it is curved and does not reach the edge of the

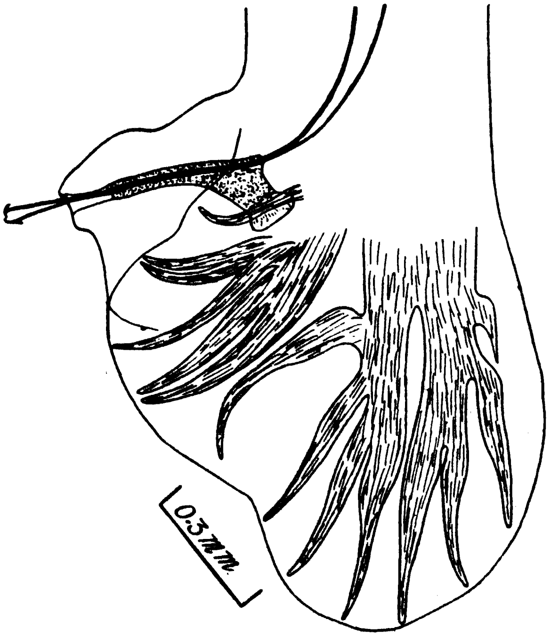


Fig. 25.—*Trichomena* (*Cylicocyclus*) *gyalocephaloides* sp. nov. Dorso-lateral view of bursa.

bursa. The postero-lateral and medio-lateral rays are more or less parallel, and the antero-lateral diverges slightly. The ventral rays are closely apposed to each other except for their tips. A large prebursal papilla is present. The genital cone is large, about 0·45 mm. long and carries two teat-like papillae 0·048 mm. long by 0·03 mm. thick at their base. A large and boot-shaped gubernaculum is present measuring 0·3 to 0·37 mm. length; its under surface (sole) is grooved to accomodate the two spicules which are long and slender and terminate in double hooked tips; they are 2·1 to 2·2 mm. long with a maximum thickness of 0·01 mm. and their terminal portions carry small alae.

The posterior extremity of the female is bent dorsalwards and a prominent swelling on its ventral surface gives it the characteristic appearance of a human foot (Fig. 26). This swelling causes a slight twisting of the " foot " towards the left side and consequently the vulva and anus come to lie on the right side of the foot. The tail is short and conical and is from 0·2 to 0·24 mm. long. The vulva is situated 0·18 to 0··24 mm. anterior of the tail tip and leads into a long and straight vagina 1·1 to 1·2 mm. long by 0·11 mm. thick with lumen 0·066 mm. in diameter; the ovejectors each measure 0·9 mm. in length and are parallel. The eggs are oval and thin-shelled and morulated *in utero;* they measure 0·089 to 0·091 mm. in length by 0·048 to 0·053 mm. across.

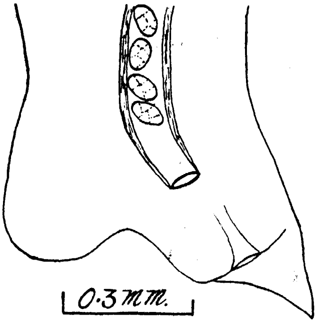


Fig. 26.—*Trichonema* (*Cylicocyclus*) *gyalocephaloides* sp. nov. Female tail.

Affinities.—The posterior hoop-like thickening of the buccal capsule and the numerous and small elements of the internal leaf crown originating from the anterior border of the buccal capsule, places this species in Ihle's sub-genus *Cylicocyclus*. However, it differs from all the members of this sub-genus, in the great delevopment of its oesophageal funnel, the shape of the terminal portion of the female body, and in that the vulva and anus are not ventral but lateral in position. Except for these points and the absence of long cephalic papillae, this species is otherwise very similar to *T.* (*Cylicocyclus*) *auriculatum* (Looss).

Specific diagnosis.—Trichoneminae reaching 14 mm. in length or longer. Head swollen; buccal capsule barrel-shaped with prominent posterior hoop; 44 external and numerous internal leaf elements. Oesophagus club-shaped, its head enlarged to accomodate a large funnel. Male bursa typical. Spicules just over 2 mm. long, slender, each terminating in a double hook. Posterior extremity of female shaped like a human foot. Vulva and anus lateral in position.

Host: Hippotigris sp.

Location: Colon.

Locality: Tanganyika.

Types in the Onderstepoort Helminthological Collection.

Trichonema (Cylicocyclus) auriculatum (Looss, 1902).

In association with the foregoing species there were six specimens of the above species consisting of three males and three females. All had unfortunately burst. This species is very similar to the foregoing but differs in having long horn-like lateral cephalic papillae and the oesophageal funnel is smaller; the posterior extremity of the female is not bent dorsalwards, but this may be due to shrinkage as a result of bursting.

Habronema numidae sp. nov.

Eight males and seven females were recovered from under the gizzard lining of guinea-fowls; they were reddish in colour and showed a tendency to become coiled into a loose spiral. The males are from 11 to 13 mm. long and the females 18 and 19 mm. long; the former attain their maximum thickness of 0·18 to 0·21 mm. just anterior of the caudal alae, and the latter are thickest (0·3 to 0·36 mm.) just anterior of the vulva.

The mouth is surrounded by four lips which are small and inconspicuous; the longest are the two lateral lips, each of which has a somewhat rectangular body with a dorsal and ventral horn-like process arising from its anterior corners (Fig. 27); its free face is slightly trilobed and it carries a small papilla towards its base. The dorsal and ventral lips are indented in their middle and carry

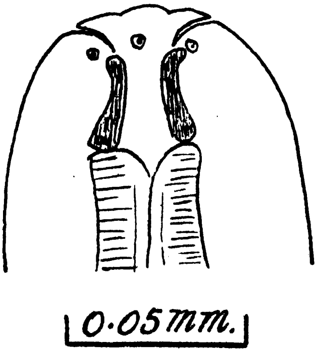
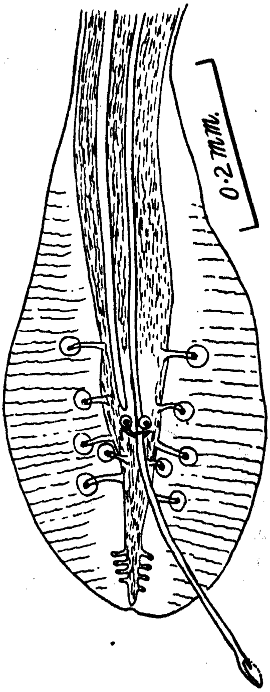


Fig. 27.—*Habronema numidae* sp. nov. Cephalic extremity.

keel-like thickenings extending into the mouth cavity on their inner surfaces; each carries two small sub-ventral pappillae. The mouth leads into a buccal capsule about 0·03 mm. deep in the males and 0·036 mm. deep in the females and having an internal diameter of 0·01 and 0·012 mm. in the two sexes respectively; its wall consists of thickened chitin which is thickest at its base. The oesophagus consists af an anterior muscular and a posterior glandular portion; the muscular portion is 0·27 to 0·3 mm. long by 0·024 mm. thick in the male and 0·33 mm. long by 0·026 mm. thick in the female. In the male the glandular oesophagus is 1·7 to 2 mm. long and in the female 2·4 to 2·6 mm. long; its maximum thickness, at its posterior end, is 0·06 and 0·065 mm for the two sexes respectively. The nerve ring is from 0·21 to 0·24 mm. from the anterior end, and the excretory pore 0·25 to 0·29 mm. from the anterior end. Cervical papillae were not seen.



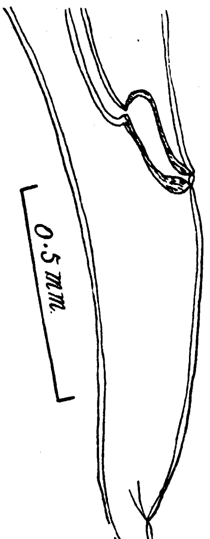


Fig. 28.—*Habronema numidae* sp. nov. Posterior extremity of male, ventral view.

Fig. 29.—*Habronema numidae* sp. nov. Female tail.

The caudal alae of the males are from 0·42 to 0·52 mm. long and the whole expansion measures about 0·275 mm. across at the level of the cloaca (Fig. 28); its posterior end is rounded. The alae show numerous wavy markings extending from its outer edge to the body wall. There are 10 pairs of caudal papillae; four pairs are lateral and pedunculate, of which two are precloacal and two post cloacal; one pedunculate pair is ventral and situated midway between the two post cloacal pedunculate papillae; a pair of sessile papillae is situated just anterior to the cloaca; the remaining four pairs are small and situated laterally towards the posterior end of the tail. The spicules are well developed and unequal; the left is 1·08 to 1·11 mm. long and has its tip slightly bent and alate; its proximal end is 0·015 mm. thick and its distal portion just anterior of the bend is 0·012 mm. thick. The right spicule is 0·42 to 0·438 mm. long, has a uniform thickness of about 0·015 mm. and ends in a bluntly rounded tip. A gubernaculum is present, measuring about 0·07 mm. long.

The posterior extremity of the female (Fig. 29) tapers off to form a blunt tail 0·126 to 0·13 mm. long. The vulva is not prominent and is located towards the posterior end, about 0·75 mm. anterior of the anus. A very short vagina leads into the somewhat pyriform vestibule about 0·25 mm. long by 0·06 mm. in diameter; a long unpaired trompe—0·78 mm. long—arises from its dorsal side and passes forwards to join the two parallel uteri. The numerous eggs are oval, thick shelled and embryonated *in utero*; they measure 0·042 to 0·045 mm. long by 0·024 mm. thick and the shell has a thickness of 0·003 mm.

Affinities.—Of the avian members of the genus *Habronema* which possess no cervical alae and in which the vulva is situated near the posterior extremity, the above described species approaches most closely the species *H. eurycerca* (Seurat, 1914) and *H. parroti* (Seurat, 1917). The arrangement of the male caudal papillae in these two species and in the writer's is practically identical and the lengths of the spicules are also very smilar. *H. eurycreca* differs from the writer's species in that the vestibule is joined to the vulva by a long neck and the lips are more complicated, and *H. parroti* differs especially in the structure of the lips which have a digitiform appearance when viewed laterally and they bear small teeth on their inner surface.

Specific diagnosis.—Spirurinae reaching a length of 19 mm. in the female and 12 mm. in the male. Four lips of which the two lateral have horn-like processes at their anterior corners. Cervical alae absent. Nine pairs stalkel papillae on male tail (two pairs precloacal) and one sessile pair on anterior lip of cloaca. Spicules unequal, 1·1 and 0·44 mm. long. Gubernaculum present. Vulva near anus. Vestibule short, no neck; common trompe long. Uteri parallel. Eggs oval and thick-shelled, 0·042 to 0·045 mm. long by 0·024 mm. thick; embryonated *in utero*.

Host: Numida mitrata Pall. and *Numida* sp.

Habitat: Under gizzard lining.

Locality: Nyasaland, Transvaal and Swaziland.

Types in the Onderstepoort Helminthological Collection.

Habronema fichueri Seurt 1916, var. *elanii* nov.

The material which the writer has determined as a variety of Seurat's species consisted of seven males and more than a dozen females; they were obtained from the stomach of a Black-shouldered Kite—*Elanus caeruleus*—shot at Onderstepoort; some of the worms were totally embedded under the mucosa.

Although this material originates from a member of the Falconidae whereas Seurat obtained his from a member of the Ardeidae, the two materials, except for a few minor points, appear to be identical. They agree in their size, structure of the lips, coarse body annulations, ornamentation of the male caudal extremity (Fig. 30), position of the vulva, structure of the female genitalia and size of the eggs. The only differences which the writer could find are that the left spicule is slightly shorter in his material (0·9 to 1·08 mm. against 1·27 mm.), the terminal hooks are placed one anterior to the other and not on either side of the left spicule as drawn by Seurat, and the writer was able to find only two labial

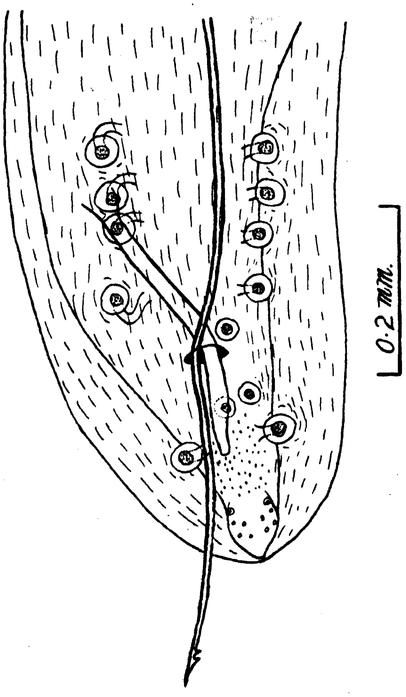


Fig. 30.—*Habronema ficheuri elanii* var. nov. Posterior extremity of male, ventral view.

teeth and not three as observed by Seurat. These differences the writer considers to be of minor importance and not of specific significance.

Host: Elanus caeruleus (Desf.).

Location: Stomach.

Locality: Pretoria district, Transvaal.

Types in the Onderstepoort Helminthological Collection.

Acuaria semei * sp. nov.

One male and ten females of this species were collected from a Giant Bustard; some of the specimens were brownish in colour whereas the remainder were cream-coloured. The body is attenuated in both sexes, the attenuation, however, being more marked in the anterior body half of the females. The male is 12 mm. long by 0·36 mm. wide just anterior of the cloaca and the females vary in length from 10 mm. to 19·5 mm. with a maximum thickness just anterior of the vulva of 0·36 to 0·4 mm. All the specimens were mature.

The cuticle is finely annulated and the mouth is bordered by two lateral triangular lips, each carrying a triangular tooth at its apex and two sub-median papillae towards their bases. Two small cervical papillae are present, situated just behind the level of the junction of the pharynx and oesophagus. Four pairs of cordons originate between the lips, two pairs emerging on their dorsal and ventral sides respectively; the cordons run in pairs and pass straight down along the submedian lines of the body to about the level or just posterior of the vulva in the female, and in the male they terminate about 0·24 mm. anterior of the cloaca.

The mouth leads into cylindrical pharynx which is generally slightly bent; it is about 0·36 mm. long by 0·036 mm. broad in the females, and 0·3 mm. long by 0·03 mm. wide in the male. The oesophagus is relatively very long reaching a length of over 5 mm. in the females, and 3·9 mm. in the male; in the former the muscular portion of the oesophagus is about 0·72 mm. long by 0·12 mm. broad at its posterior end, and in the latter it is 0·54 mm. long. The nerve ring encircles it near its anterior extremity. The glandular oesophagus reaches a length of 4·68 mm. in females and is 3·36 mm. long in the male; its initial portion is 0·24 and 0·2 mm. thick at its anterior extremity and 0·266 and 0·23 mm. broad at its posterior end in the two sexes respectively. The excretory pore is situated about 0·25 mm. behind the level of the nerve ring in both sexes.

The posterior extremity of the male is spirally coiled and terminates in a conical tail 0·4 mm. long.. Caudal alae are practically absent (Fig. 31). There are four pairs of symmetrically placed sessile precloacal papillae and six pairs of sessile postcloacal papillae; the posterior three pairs of these are arranged slightly closer to each other than the anterior three pairs. The two spicules are massive and slightly unequal; the left is 0·354 mm. long by 0·32 mm. wide at its base and the right is 0·24 mm. long and 0·045 mm. mide and terminates in a broad, rounded and alate tip. A gubernaculum is absent.

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* "Seme" is the Zulu name for the Giant Bustard.

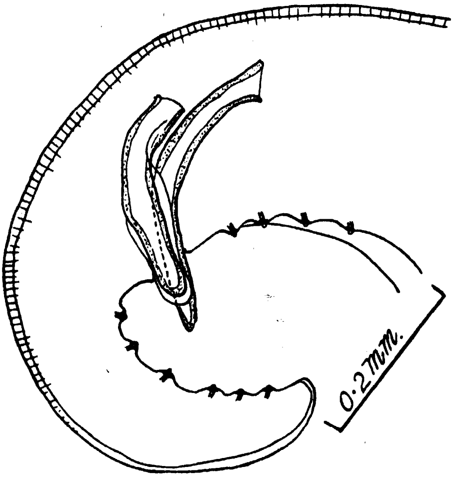

Fig. 31.—*Acauria semei* sp. nov. Posterior extremity of male, lateral view.

In the females the vulva is situated towards the posterior end of the body, about 1·6 to 2 mm. from the anus (Fig. 32); it leads into an elongate vagina 1·0 to 1·2 mm. long by 0·12 to 0·14 mm. thick, which is more or less straight and passes obliquely forwards. The uteri extend forwards and are filled with numerous smooth, oval and embryonated eggs measuring 0·043 to 0·045 mm. long by 0·024 to 0·026 mm. broad. The tail is conical, about 0·26 mm. long and is sharply bent dorsalwards, giving the end of the body a hooked appearance.

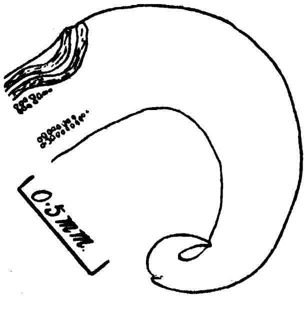

Fig. 32.—*Acuaria semei* sp. nov. Posterior extremity of female.

Affinities.—The great length of the cordons distinguishes this species from all the known members of this genus except from *A. lata* Maplestone, 1931. Maplestone's species differs, however, in that the cordons do not extend quite so far back, being about three-quarters of the body length, and its vulva is situated just behind the middle of the body.

Specific diagnosis.—Acuariinae reaching a length of 12 mm. in the male and 19·5 in the female. Four pairs of very long cordons, extending to near the cloaca and vulva in the two sexes respectively. Caudal alae practically absent in the male. Four pairs precloacal and six pairs postcloacal papillae, all sessile. Spicules short and stout, right spicule 0.24 mm. long and terminates in a broad rounded tip; left spicule thinner, 0·354 mm. long. Vulva near posterior end, about two mm. from tail tip. Vagina straight, passes obliquely forwards. Uteri directed anteriorly. Tail bent sharply dorsalwards. Eggs oval, smooth and embryonated *in utero.*

Host: Choriotis kori (Burch.)

Location: Under gizzard lining.

Locality: Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

Cheilospirura gruveli (Gendre, 1913) Cram, 1927.

This species was represented by two males and one female recovered from under the gizzard lining of a francolin (*Pternistes swainsoni*) (A. Smith) shot at Onderstepoort. The female was 32 mm. long, its cuticular cordons 0·72 mm. long and its eggs measured 0·036 by 0·023 mm.; the two males were 10 and 11 mm. long and their spicules measures 0·6 and 0·12 and 0·53 and 0·144 mm. for the left and right respectively. In all other respects the writer's material agreed with the data given by Cram (1927).

Histiocephalus choriotidis sp. nov.

Three mature specimens of this species were recovered from under the gizzard lining of a Giant Bustard; unfortunately only females were present.

The specimens are brownish red and are attenuated towards both extremities; two of the specimens were each 12 mm. long by 0·36 and 0·39 mm. thick respectively, the third specimen was too coiled for measuring.

The mouth is bounded by four lips, the two laterals being relatively large and rectangular with their free margin indented, and the dorsal and ventral being small and pointed (Fig. 33); the lateral lips are about 0·03 mm. high and 0·09 mm. broad; posteriorly each carries a chitinous flange which is cut up to form six appendages each terminating in one to four projections; each appendage is from ·042 to 0·045 mm. long. Each dorsal and ventral lip carries two large papillae lateral and behind their bases. A constriction encircles the body behind the head, and immediately posterior to this groove the cuticle is inflated to form a collar about 0·075 mm. long by 0·165 mm. across; the collar is supported by a number of external longitudinal cuticular ridges. The mouth leads into a pharynx which is laterally compressed; it is about 0·06 mm. deep, its internal diameter about 0·027 mm. and its cuticular wall has a thickness of about 0·003 mm. No teeth were observed on the inner surface of the lips.

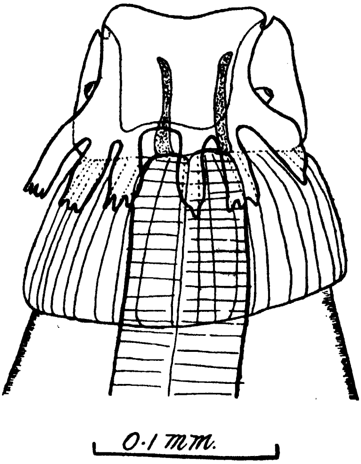

Fig. 33.—*Histiocephalus chariotidis* sp. nov. Anterior extremity.

The oesophagus is relatively long and consists of an anterior muscular and a posterior glandular portion; the former is about 0·54 mm. long and increases in diameter from 0·06 to 0·078 mm.; the nerve ring encircles it about 0·22 mm. behind its anterior end; the glandular oesophagus is from 3·5 to 3·7 mm. long by 0·12 to 0·2 mm. in diameter.

The vulva is a rounded non-protuberant aperture situated in the oesophageal region, 2 to 2·25 mm. from the anterior end; the details of the vagina and uteri could not be made out as they were obscured by the numerous eggs; the initial portion of the vagina has a diameter of 0·07 mm. and the remaining portion of it appears to be long and undifferentiated. The eggs are oval, smooth, thick-walled end embryonated *in utero*; they measure 0·054 to 0·057 mm. long by 0·039 mm .thick and the shell has a thickness of nearly 0·003 mm. The tail is short and conical and is from 0·1 to 0·12 mm. long.

Affinities.—Only two species of nematode have been assigned to this genus with certainty, namely *H. laticaudatus* (Rud., 1819) and *H. tridens* Gendre, 1912, both having been recovered from Otidiform birds. The female of the species described above appears to be closely related to both these species, but differs from the former in having 12 cuticular appendages instead of 20 to 24 and from the latter in that the lips are not trilobed and the appendages do not terminate in single points.

Specific diagnosis.—Schistotrophinae reaching 12 mm. in length, possessing six cuticular appendages behind each lateral lip, each appendage terminating in one to four points. Oesophagus over one-third of total body length. Vulva in oesophageal region 2·0 to 2·5 mm. from anterior end. Tail short and bluntly conical.

Host: Choriotis kori (Burch.)

Location: Under gizzard lining.

Locality: Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

*Trichuris vondwei** sp. nov.

One of two cane rats, sent alive to this institute, was on post mortem found to harbour five male and six female specimens of the above species; only two of the females appear to be mature and contain fully developed eggs.

The thickened portion in the males is from 15 to 16 mm. long with a maximum thickness of 0·336 mm. for the smallest to 0·432 mm. for the largest specimens; in the two mature females these measurements are respectively 16 and 20 mm. in length by 0·58 and 0·62 mm. in breadth. The posterior extremity of the male is bluntly rounded and carries a cone-like papilla on either side (Fig. 34); in the female the posterior end is pointed.

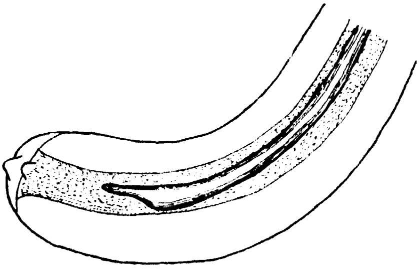

Fig. 34.—*Trichuris vondwei* sp. nov. Posterior extremity of male.

The spicule varies in length from 1·98 to 2·58 mm. and is completely retracted in all the males; its proximal end, just behind its head, has a diameter of 0·057 to 0·069 mm.; posteriorwards it becomes thinner until about 0·1 mm. from its tip it has a thickness of 0·02 to 0·025 mm.; immediately posterior it enlarges to a thickness of 0·03 to 0·035 mm. and then suddenly becomes narrower to form a bluntly rounded tip 0·06 to 0·07 mm. long; this sudden narrowing of the spicule tip gives the spicule a characteristic shape which is constant in all five the males. The spicular sheath is retracted in all the males, and in this position is from 0·78 to 0·96 mm. in length; it is densely covered by fine spines which are longest at its distal end, i.e. if the spicule was fully everted the largest spines would be nearest the body and the smallest away from the body. The cloaca varies in length from 1·8 to 2·27 mm. and is straight; the large spicular tube enters it about 0·6 to 0·66 mm. from its proximal end; the distance from the proximal end of the spicular sheath to the entrance of the spicular tube into the cloaca varies from 0·43 to 0·69 mm. The ejaculatory duct is large and straight and measures from 6·5 to 8·14 mm. in length; a slight narrowing forms the junction between it and the vas deferens, whose wall is much thinner and lumen larger; the vas deferens passes forwards, forming kinky loops, to just behind the level of the end of the oesophagus; in this kinked condition it occupies 4·6 to 6·3 mm. of the body length; the testis, which pass backwards dorsal of and parallel to the vas deferens and ejaculating duct, is thrown into closely packed kinks up to about 0·15 mm. from its distal end; this end position is straight and lies opposite the proximal portion of the cloaca.

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* "Vondwe" is the Zulu name for the Cane Rat.

In the two mature females the vagina passes backwards for 6·9 and 7·1 mm. respectively; it is wavy and its lumen carries no spines, but the cells lining its lumen are spike-like and these increase in robustness towards its posterior end; at its opening the lumen has a diameter of 0·06 mm., about one-third of its length down a diameter of 0·084 mm. and at its posterior end its diameter is 0·36 mm. A constriction joins the vagina to the smooth walled uterus which extends to the posterior end of the body and is filled with eggs.

The eggs are oval and thick-shelled, and measures 0·06 to 0·66 mm. in length, including the plugs, by 0·032 to 0·033 mm. in breadth.

Affinities.—As the peculiar shape of the spicule tip was present in all the five males, this character can be taken to be of specific significance. This shape of the spicule tip plus the kinked nature of the vas deferens, the presence of two caudal papillae in the male and the simple but wavy nature of the vagina separate this species from all the known whip-worms from rodents and ruminants in which these organs have been described. Caudal papillae are also present in *T. gazellae* Gebauer, 1933, from Damagazelles, *T. muris* (Schrank, 1788) from various rodents and *T. parvispiculum* Ortlepp, 1937 from Goats, but in the former species the spicule is much longer (4·15 mm.) and in the second and last species much shorter (0·76 mm. and 0·85 to 1·07 mm. respectively).

Specific diagnosis.—Trichuridae with the thickened portion of the body up to 16 mm. long in the male and 20 mm. long in the female. Spicule well chitinized and 1·98 to 2·58 mm. long; tip cut out on one side and bluntly pointed. Two caudal papillae, one on either side of cloaca. Cloaca 1·8 to 2·27 mm. long and spicular tube enters it about 0·6 mm. from its proximal end. Spicular sheath with numerous small spines. Ejaculatory duct straight 6·5 to 8·4 mm. long. Vas deferens kinked and occupies 4·6 to 6·3 mm.

of body. Vagina simple and wavy, with no spines but cells lining lumen are spike-like. Eggs 0·6 to 0·66 mm. long, including plugs, by 0·032 to 0·033 mm. broad.

Host: *Thyronomys swinderianus variegatus* Ptrs.

Location: Large intestine.

Locality: Zululand, Natal.

Types in the Onderstepoort Helminthological Collection.

RESUME.

In the preceeding pages the writer discusses twenty species of helminths of which fourteen are described as new. These are *Hymenolepis cormoranti* sp. nov and *Contracaecum carlislei* sp. nov. from Reed Cormorants: *Idiogenes kori* sp. nov., *I. kolbei* sp. nov. *Subulura otidis* sp. nov., *Acuaria semei* sp. nov., and *Histiocephalus choriotidis* sp. nov. from a Giant Bustard; *Habronema numidae* sp. nov. from a guinea fowl; *Habronema ficheuri* var. *elanii* nov. from a Black-shouldered Kite; *Porrocaecum spathulispiculum* sp. nov. from an unidentified bird; *Raillietina (S.L.) thryonomysi* sp. nov. and *Trichuris vondwei* sp. nov. from Cane Rats; *Cooperiodes hepaticae* sp. nov. from an Impala and *Trichonema (Cylicocyclus) gyalocephaloides* sp. nov. from a Zebra.

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Section III.

Mineral Metabolism and Deficiency.

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Sulphur Metabolism. VI.—The Effect of Various Levels of Fats and Proteins in the Ration on the Toxicity of Elementary Sulphur.

By J. H. KELLERMANN, Section of Biochemistry, Onderstepoort.

LEWIS AND LEWIS (1927) have found that the incorporation of one per cent. elementary sulphur in the milk powder-starch diet of Sherman and Merrill (1925) and in the low protein diet of Osborne and Mendel was definitely toxic. With a few exceptions, the livers of the rats which died showed a marked peripheral zonal necrosis. However, these authors found that the rats on the Osborne-Mendel diet were more resistant to the toxic effects of sulphur than were those on the Sherman-Merrill ration. The former diet is richer in fats and proteins than the latter and the authors expressed the opinion that the lower toxicity of sulphur in the Osborne-Mendel diet might be due to its higher fat and protein contents. Their suggestion is that the fat probably forms a coating around the sulphur particles which either prevented them from coming into intimately close contact with the intestinal mucosa or the bacterial action on them is thereby reduced. Furthermore, the higher protein level might lead to a better state of nutrition and hence a greater resistance to the toxic action. This supposition, as far as the writer is aware, has never been put to the test, and it was the object of the following experiments to determine its validity.

EXPERIMENTAL.

Young rats of the Wistar strain were used in these experiments. They were equally divided amongst the various groups with respect to litter, sex and weight. Each animal was kept in a separate cage on a raised screen bottom. In addition to the distilled water, to which the animals always had free access, each rat was fed daily its particular ration *ad libitum* with recorded food consumption for the experimental period. Owing to the slightly acid nature of the yeast extract on starch, to be described later, the diets rapidly developed a rancid odour. In order, therefore, to prevent rancidity in these diets, the yeast extract was withheld from the bulk of the diet and intimately mixed with each day's supply as practised by Gallup and Reder (1935). The composition of the rations is given in Table I.

The basal diets contained all the dietary essentials known. The vitamin B complex was given in the form of an extract prepared from brewers' yeast according to the method of Itter, Orent and

TABLE I.

Composition of Rations in Percentage by Weight.

	Low Protein.	High Protein.	Low Fat.		High Fat.	
" Vitaminized starch "	10	10	10	10	10	10
Dextrinized starch	44·7	15	49	43·6	31·4	26
Casein	10	40	20	20	20	20
Sucrose	12	12	12	12	12	12
Salt 40	4·5	4·5	4·5	4·5	4·5	4·5
Agar	2·5	2·5	2·5	2·5	2·5	2·5
Cod-liver oil	1	1	2	2	2	2
Elementary sulphur	3	3	—	3	—	3
Lard	—	—	—	2·4	17·6	20
Butter fat	12	12	—	—	—	—
l-cystine	0·3	—	—	—	—	—

McCollum (1935). This extract was evaporated on dextrinized starch subsequently designated " vitaminized starch ". One gram of this starch was equivalent to 0·5 g. of yeast. The composition of Salt 40 was similar to that of Steenbock and Nelson (1923) as modified by Keenan and others (1933). However, in order to obtain a sulphur-free salt mixture the magnesium and manganese sulphates were replaced by equivalent amounts of their carbonate forms and the $CuSO_4 \cdot 5H_2O$ by cupric acetate. In addition to lard the effects of butterfat, beef dripping (M.P. 43-49° C.) and olive oil on the toxicity of elementary sulphur were studied. The rations containing these fats were identical in every respect to the lard ration except that the latter was replaced by an equivalent amount of these various fats. Furthermore, all the high fat diets with and without the addition of elementary sulphur were made equicaloric by replacement of some of the starch by isodynamic quantities of fat.* Similarly, the low fat diets, with and without added sulphur, were made isocaloric and the same applied for the low and high protein rations. After the rats had been on experiment for eight weeks they were killed by a blow on the head and various organs immediately removed and preserved in a formalin solution for histopathological examination.

The results obtained on the low and high protein rations are presented in Tables II, III, and IV. Table II gives the original results, Table III the means and Table IV the analysis of variance. From Table IV it is clear that the growth-promoting value of the low protein ration was significantly greater than that of the high protein one; the probability P being ·01. This observation substantiates, therefore, the results of Osborne, Mendel and Ferry (1919), Mitchell (1923-24), St. John and others (1934), Chick and co-workers (1935) and Basu, Nath and Mukherpee (1937). However, the growth-promoting values for males and females were not significantly different and there was no interaction of sex with the protein content of the ration.

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* The energy values of the diets were calculated on the basis of 4·0 Cal. per gram for the carbohydrates and casein, and of 9·0 Cal. per gram for the various fats and oils.

TABLE II.

The growth-promoting values of sulphur supplemented rations low and high in protein content as determined by the method of Osborne, Mendel and Ferry (1919).

PERIOD : 8 WEEKS.

Ration.	Sex.	Total Food consumed.	Protein consumed. P.	Weight increase. W.	Ratio $^{W}/_{P}$ (or G–P value).
		g.	g.	g.	
Low Protein (8·5 per cent.)..	Male	363	30·85	69	2·24
	Male	265	22·52	27	1·20
	Male	396	33·66	76	2·26
	Female	371	31·54	72	2·28
	Female	322	27·37	41	1·50
	Female	292	24·82	34	1·37
High Protein (34 per cent.)....	Male	316	107·4	64	0·06
	Male	417	141·8	106	7·75
	Male	298	101·4	59	0·58
	Female	321	109·1	66	0·60
	Female	302	102·7	45	0·44
	Female	283	96·23	40	0·41

TABLE III.

Mean Growth-promoting Values.

	Males.	Females.	Total.
Low Protein..................................	1·9	1·72	1·81
High Protein..................................	0·64	0·48	0·56
TOTAL..............................	1·27	1·1	1·19

TABLE IV.

Analysis of Variance.

	D.F.	S.S.	M.S.	S.D.	Log_e S.D.	Z.
Low protein *vs*, high protein	1	4·6501	4·6501	2·156	0·7682	1·6895
Males *vs*. females......	1	0·0884	0·0884	—	—	—
Interaction............	1	0·0004	0·0004	—	—	—
Error.................	8	1·2578	0·1582	0·398	−0·9213	—
TOTAL..........	11	5·9967				

From Fisher's (1932) Table of "z":

		at $p = \cdot05$	at $p = \cdot01$
For $n_1 = 1$ $n_2 = 8$	$z =$	0·8355	1·2106

Hence this value of z is significant at $P = \cdot01$.

The results obtained on the low and high fat rations are given in Tables V and VI.

TABLE V.

Effect on Efficiency Quotient of adding 3 per cent. Elementary Sulphur to Synthetic Rations of Various Fat and Protein Content. Period 8 Weeks.

Ration.	Supplemented with.	Number of Rats.	Sex.	Mean Gain.	Mean total food.	Mean efficiency quotient.
Low fat	—	3	♂	143·5	670·5	3·2
Low fat	—	3	♀	98	673·5	5·41
Low fat	Sulphur	3	♂	102	554	4·65
Low fat	Sulphur	3	♀	46·8	383·6	6·29
High fat (lard)	—	6	♂	107·7	402·6	2·88
High fat (lard)	—	6	♀	85·5	395·7	3·88
High fat (lard)	Sulphur	3	♂	66	355	4·4
High fat (lard)	Sulphur	3	♀	55·7	306·3	5·46
High fat (butter)	Sulphur	3	♂	82	347	3·45
High fat (butter)	Sulphur	3	♀	66	324·3	4·62
High fat (dripping)	Sulphur	3	♂	83	352·3	3·63
High fat (dripping)	Sulphur	3	♀	64·7	331·2	4·91
High fat (olive oil)	Sulphur	3	♂	72·3	312·7	3·87
High fat (olive oil)	Sulphur	3	♀	59·3	313·5	5·25
All males	—	24	—	93·8	427·7	3·62
All females	—	24	—	68	389·7	4·96
All low fat diets	—	12	♂ & ♀	97·6	570·4	4·89
All high fat diets	—	36	♂ & ♀	74·2	344·1	4·10
Low fat diets	− Sulphur	6	♂ & ♀	120·7	672	4·30
Low fat diets	+ Sulphur	6	♂ & ♀	74·4	468·8	5·47
High fat diets	− Sulphur	12	♂ & ♀	96·6	399·1	3·38
High fat diets	+ Sulphur	24	♂ & ♀	68·6	330·3	4·45

Table V gives the mean values and Table VI the analysis of variance of the efficiency quotients. The data in the latter table show that the efficiency quotients, calculated according to the method of Palmer and Kennedy (1929)* of the males were significantly better than those of the females. The probability P was ·01 which substantiates the results of Palmer and Kennedy (1931). On the high fat rations efficiency quotients were obtained that were significantly superior (P = ·01) to those on the low fat rations. This result might be expected in view of the great difference in caloric value between the two types of diet. Within both low and high fat rations the addition of elementary sulphur significantly lowered the animals' efficiency to utilize their food; the probability P being ·01. The lower food consumption on the sulphur rations might have contributed to the inferior utilization of food inasmuch as Palmer and Kennedy (1931) found that a restriction of food intake lowered the index of food utilization. However, the efficiency quotients on the butterfat, lard, dripping and olive oil rations were not significantly different, and the same was true for the interaction of sex with the various fats.

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* The dry matter only, not the digestible dry matter, was used in these calculations.

TABLE VI.

Analysis of Variance of Efficiency Quotients.

	D.F.	S.S.	M.S.	S.D.	$\log_e$ S.D.	Z.
Male *vs.* female.......	1	21·6008	21·6008	4·648	1·5364	2·0540
Low fat *vs.* high fat...	1	5·6486	5·6486	2·377	0·8658	1·3834
Within low fat: Sulphur *vs.* no S.	1	4·0950	4·0950	2·024	0·7051	1·227
Within high fat: S. *vs.* no S.	1	9·1022	9·1022	3·017	1·1041	1·627
Within S. butter *vs.* lard *vs.* dripping *vs.* olive oil	3	2·6819	0·8940	0·946	—·0555	0·4621
Between all fats.......	6	21·5277	3·5879	1·894	0·6387	1·1563
Interaction of sex with fats	6	1·8009	0·3002	0·548	—	—
Treatments...........	13	44·9294	3·4561	1·859	0·6201	1·1377
Error.................	34	12·0773	0·3552	0·596	—0·5176	—
TOTAL....	47	57·0067				

From Fisher's (1932) Table of z:

		at P = 0·5	at P = ·01
for n_1 = 1,	n_2 = 30,	z = 0·7141	1·0116
n_1 = 3,	n_2 = 30,	z = 0·5632	0·7531
n_1 = 6,	n_2 = 30,	z = 0·4420	0·6226
n_1 = 12,	n_2 = 30,	z = 0·3691	0·5224

A comparison of the z values from the above table, with these criterions indicate that all, except the " Butter *vs.* lard *vs.* dripping *vs.* olive oil " z, are significant at P = ·01.

On the whole, the histopathological changes noted in the livers of rats fed the various diets, with and without the addition of elementary sulphur, did not vary much from those seen in the livers of normal animals. The most common observations were fatty changes which varied from slight to fairly advanced and diffuse. The kidneys of the animals on the low and high protein rations were also examined. However, except for slight fatty changes, these organs did not show any constant pathological changes, and one is therefore led to the conclusion that elementary sulphur has no deleterious effects on the structure of livers and kidneys, which is most probably also true for all other organs of rats as long as the animals are fed a ration that contains all the essential constituents for growth.

SUMMARY.

1. The extent to which various levels of fats and proteins in rations could influence the effects of elementary sulphur on rats as judged from growth, food utilization and pathological lesions, was studied.

2. The growth-promoting value (gain per unit protein consumed) of the low protein ration was significantly greater than that of the high protein diet.

3. On the same ration the males utilized their food significantly better than the females.

4. Within both low and high fat rations the addition of elementary sulphur significantly lowered the animals' efficiency of food utilization.

5. Irrespective of the fat and protein contents of the diets the livers and kidneys of the sulphur fed rats, as compared with those of the control groups, did not exhibit any constant pathological changes.

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Sulphur Metabolism. VII.—The Effect of the Acid-Base Balance of the Ration on the Toxicity of Elementary Sulphur.

By J. H. KELLERMANN, Section of Biochemistry, Onderstepoort.

ELEMENTARY sulphur, when absorbed by the animal organism, is eventually excreted in the urine primarily, if not entirely, as sulphates. [Denis and Reed (1927), and Kellermann (1935)]: Evidently then, through the oxidation of sulphur, a large amount of acid is formed in the system. For instance, results (unpublished) obtained at this Institute show that 50 per cent., and even more, of elementary sulphur, when incorporated in the rations at a three per cent. level, can be absorbed. On this basis the sulphur in every 100 g. of food can give rise to about 936 c.c. of 0·1 N. acid which, together with the acid- and base-forming elements in the rest of the food will naturally determine the ultimate acid-base balance of the diet.

From the results of various investigators [Goss and Schmidt (1929), Mitchell and Miller (1931), and Lamb and Evvard (1932] it appears that the growth and reproduction of rats and pigs are not materially affected by diets with strongly acid-forming potentialities. Furthermore, Steenbock, Nelson and Hart (1914) observed that calves can tolerate large amounts of acid (220-500 c.c. N.HCl) without any ill effects. Similar results were obtained with dairy cows.

On the other hand, Addis, Mackay and Mackay (1926-27) found that by adding 2 grams of $CaCl_2$ to every 98 grams of control diet " the acid diet rats at first grew almost but not quite as quickly as the controls but from about the 120th day of age they ceased to gain and thereafter tended slowly to lose in weight. The food graph reveals the paradoxical circumstance that not only is this not to be explained by the insufficient food supply but that on the contrary the inhibition of growth and failure of maintenance occurs in spite of a food intake which considerably exceeds that of the controls." Similarly, Comes (1934), (1936) observed that the administration of not only acid but also alkaline diets caused a retardation in the growth of rats. Takahashi (1936) found that the feeding of sulphur to rats, pigeons, rabbits, guinea-pigs and frogs, for 2 to 3 months, produced severe acidosis and typical calcium metastases.

If, then, a diet is already potentially acid, which is likely and no doubt often the case in the nutrition of pigs, dogs and human beings, the incorporation of elementary sulphur may so enhance the potential acidity of the ration that it may prove detrimental to the growth and well-being of the individual. Therefore, in order to test this assumption experiments were conducted in which the toxicity of elementary sulphur in relation to the potential acidity and alkalinity of the ration was studied.

EXPERIMENTAL.

Young white rats were used in these experiments. The experimental procedure was the same as previously described (Kellermann, 1938, 2) with the only difference that the food was given in a moist instead of a dry form. In some groups, as shown in Table III, the food was moistened with fresh orange juice and in others with distilled water. Orange juice was selected on account of its alkalinizing properties as shown by Saywell and Lane (1933), Schuck (1934) and Clouse (1935). Furthermore, it was considered of interest to ascertain whether plant juices, with orange juice as representative, possess any specific properties in modifying the effects of sulphur as judged by the growth and other performances of the animals. In each case 2 c.c. of fluid was added to every gram of food and mixed thoroughly. Enough orange juice to last for a few days was squeezed out, strained and kept in a refrigerator. The dry matter of each batch so prepared was determined immediately and a record kept of the interval each batch was fed. The total amount of orange juice (dry) consumed added to that of the corresponding ration eaten constituted the total food intake for the experimental period.

Inasmuch as the results in the literature are not incontrovertible with respect to the effects of acid- and base-forming diets on growth and structure, it was necessary to feed such diets with and without the addition of sulphur in order to be able to distinguish between the effects of sulphur and those of the basal diets as such. The composition of the basal rations is given in Table I.

TABLE I.

Composition of Rations in Percentage by Weight.

	Acid-forming Diet.		Base-forming Diet.	
" Vitaminized starch "	10	10	10	10
Dextrinized starch	39·5	34·1	33·2	27·8
Casein	20	20	20	20
Sucrose	12	12	12	12
Butter fat	12	14·4	14·8	17·2
Agar	2	2	2	2
Cod-liver oil	1	1	1	1
Elementary sulphur	—	3	—	3
Acid salts	3·5	3·5	—	—
Basic salts	—	—	5	5
Sodium bicarbonate	—	—	2	2

The " vitaminized starch " was prepared as described previously (Kellermann, 1938, 2) and the composition of the acid and basic salt mixtures is given in Table II. The acid and base equivalents of the basal acid- and base-forming diets were 316·6 c.c. N/10 acid and 235·5 c.c. N/10 alkali, respectively, as determined by the method of Davidson and LeClerc (1935).

TABLE II.

*Composition of Acid and Basic Salt Mixtures.**

	Acid Salt Mixture.	Basic Salt Mixture.
$CaCO_3$	—	1·627
Na_3PO_4	—	2·781
$CaHPO_4.2H_2O$	2·8	—
$NaCl$	0·3	0·3
KCl	0·2	0·2
$MgCl_2.6H_2O$	0·16	—
Iron citrate	0·08	0·08
$MgCO_3$	—	0·0662
$MnCl_2$	0·0058	0·0058
KI	0·0023	0·0023
$ZnCl_2$	0·0007	0·0007
$CuCl_2.2H_2O$	0·0005	0·0005
TOTALS	3·5493	5·0635

* Roughly 3·5 g. of the acid salt mixture is equivalent to 5·0 g. of the basic mixture in so far as the metals, except sodium, are concerned.

TABLE III.

Comparison of Efficiency Quotients of Rats fed Acid- and Base-Forming Diets with and without the addition of 3 per cent. Elementary Sulphur and Orange Juice. Period, 8 Weeks.

Ration.	Supplemented with.		Number of Rats.	Sex.	Mean Gain.	Mean total Food.	Mean Efficiency Quotient.
					g.	g.	
Acid	—	—	3	♂	89·3	464·3	4·78
Acid	—	—	3	♀	61·3	401·3	7·26
Acid	—	Orange juice	3	♂	97·3	453·0	4·31
Acid	—	Orange juice	3	♀	72·3	447·0	6·39
Acid	Sulphur	—	3	♂	79·7	392·3	4·85
Acid	Sulphur	—	3	♀	45·7	324·3	9·14
Acid	Sulphur	Orange juice	3	♂	82·7	412·7	4·90
Acid	Sulphur	Orange juice	3	♀	64·3	432·9	7·33
Alkaline	—	—	3	♂	118·3	494·3	3·35
Alkaline	—	—	3	♀	68·7	416·0	6·06
Alkaline	—	Orange juice	3	♂	129·3	557·0	3·43
Alkaline	—	Orange juice	3	♀	80·3	446·8	5·57
Alkaline	Sulphur	—	3	♂	91·3	427·0	4·26
Alkaline	Sulphur	—	3	♀	60·7	383·2	6·63
Alkaline	Sulphur	Orange juice	3	♂	119·3	540·0	3·69
Alkaline	Sulphur	Orange juice	3	♀	70·7	421·5	6·26
All acid diets			24	♂ & ♀	74·1	416·0	6·12
All alkaline diets			24	♂ & ♀	88·6	460·7	4·91
All sulphur diets			24	♂ & ♀	76·8	416·7	5·88
All no-sulphur diets			24	♂ & ♀	89·6	459·9	5·15
All no-orange juice diets			24	♂ & ♀	76·9	412·8	5·79
All orange juice diets			24	♂ & ♀	89·5	463·9	5·24
All females			24	♀	65·5	409·1	6·83
All males			24	♂	100·9	467·6	4·20

All four diets were made equicaloric by replacement of some of the starch by isodynamic quantities of fat.* After the rats had been on experiment for 8 weeks they were killed by a blow on the head and various organs immediately removed and preserved in a formalin solution.

TABLE IV.

Analysis of Variance of Efficiency Quotients.

		D.F.	S.S.	M.S.	S.D.	Log$_e$ S.D.	Z.
Treats 15 D.F.	Acid or Alkaline...........	1	—‡	17·6540	4·202	1·435	1·282††
	Sulphur *vs.* no Sulphur.....	1	—	6·5940	2·559	0·940	0·787†
	Orange juice *vs.* no orange juice	1	—	3·7130	1·927	0·656	0·503
	Male *vs.* female............	1	—	83·2924	9·126	2·212	2·059††
	First Order Interactions.....	—	—	—	—	—	—
	Acidity *vs.* sulphur.........	1	—	0·2067	—	—	—
	Acidity *vs.* orange juice.....	1	—	0·5830	—	—	—
	Acidity *vs.* sex.............	1	—	0·4200	—	—	—
	Sulphur *vs.* orange juice.....	1	—	0·1622	—	—	—
	Sulphur *vs.* sex.............	1	—	0·9605	—	—	—
	Orange juice *vs.* sex........	1	—	1·3101	—	—	—
	Second Order Interactions—		Up to this point the S.S. total = 115. Since all Treats S.S. = 117·3, only 2·3 is left for the remaining 5 D.F. Even if all this is allotted to a particular D.F. it would be insignificant. The remaining S.S. need therefore not to be calculated and apportioned.				
	Acid *vs.* Sulphur *vs.* orange juice	1					
	Acid *vs.* Sulphur *vs.* sex.....	1					
	Acid *vs.* orange juice *vs.* sex..	1					
	Sulphur *vs.* orange juice *vs.* sex	1					
	Third Order Interactions—						
	Acid *vs.* Sulphur *vs.* orange juice *vs.* sex	1					
	Error	32	42·1010	1·3581	1·165	0·153	
	Total	47	159·4498				

From Fisher's (1932) Table of *Z* :—

	At P = ·05	AT P = ·01.
For $n_1 = 1$, $n_2 = 30$.	Z = 0·7141	1·0116

And hence the significance of the Z's is as above.

† Denotes significance at P = ·05. †† Denotes significance at P = ·01.

‡ These S.S. values are the same as the M.S. values in the next column since D.F. in each is unity. They have only been omitted to avoid duplication.

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* The energy values of the diets were calculated as described in the previous article.

The results obtained on the acid and alkaline rations are given in Tables III and IV. Table III gives the mean values and Table IV the analysis of variance of the efficiency quotients. The data in the last table show that the efficiency quotient, calculated according to the method of Palmer and Kennedy (1929),* of the males was significantly superior to that of the females; the probability P being ·01. Similarly, the efficiency index on the base-forming rations was significantly better than that on the acid-forming ones at probability P = ·01. Consequently this observation substantiates the results of various authors already quoted in the introduction. Furthermore, the efficiency quotient on the basal rations was significantly better than that on the diets supplemented with elementary sulphur; the probability P being ·05. The lower food consumption on the sulphur rations might have contributed to the inferior utilization of food inasmuch as Palmer and Kennedy (1931) found that a restriction of food intake lowered the index of food utilization.

Likewise, even greater effects on the efficiency index were produced by sulphur when added to synthetic rations of various fat content (Kellermann, 1938, 2). However, the addition of sulphur to a stock ration had no such influence on food utilization no matter whether equalised food consumption (Kellermann, 1936) or *ad libitum*, recorded food consumption (Kellermann, 1938, 1) was practised and it seems, therefore, that the stock ration was more effective in counteracting the detrimental effects of sulphur on food utilization and subsequent growth than the synthetic diets used under the experimental conditions.

There was no significant difference between the diets with and without orange juice. The same held for all interactions and by stating, for example, that there was no significant interaction of the acid-base balance with sulphur, is meant that the difference between acid- and base-forming rations is relatively the same in the presence or absence of sulphur.

Histopathological examination of the livers showed no unusual changes no matter whether the acid- and base-forming diets were supplemented with elementary sulphur and orange juice or not. The fatty changes noted varied somewhat in degree and distribution not only in the various diet groups, but also in individual rats kept on the same diet. However, these changes did not differ much from those encountered in the livers of rats fed a normal ration, and it can, therefore, be concluded that, under the experimental conditions, the feeding of acid- and base-forming diets, with and without the addition of elementary sulphur, had no ill-effects on the cell structure of the liver.

SUMMARY.

1. Data are presented on the relation of the acid-base balance of the ration to the effects of elementary sulphur on food utilization and the well-being of rats.

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* The dry matter only, not the digestible dry matter, was used in these calculations.

2. The efficiency quotient on the base-forming rations was significantly better than on the acid-forming ones, and the addition of elementary sulphur to these rations significantly lowered the efficiency index on both base- and acid-forming diets.

3. The efficiency quotient of the males was superior to that of the females.

4. No interaction between diets or between sex and diets appeared to be significant.

5. No pathological changes could be found in the livers of rats fed the various acid- and base-forming rations no matter whether they were supplemented with elementary sulphur or not.

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Sulphur Metabolism. VIII.—The Effect of Incomplete Rations on the Toxicity of Elementary Sulphur.

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IN a previous investigation the author (1938, 1) found that, although the incorporation of elementary sulphur in a stock ration slightly reduced food consumption and growth, it had no effect on food utilization. Furthermore, the feeding of sulphur had no appreciable influence on the cell structure of internal organs such as the liver and kidneys. This was true no matter whether the rations differed widely in their acid- and base potentialities (Kellermann, 1938, 3) and in their fat and protein contents (Kellermann, 1938, 2) and the findings do not support, therefore, the observations of Lewis and Lewis (1927) who found a greater resistance in rats to sulphur poisoning when fed the Osborne-Mendel low cystine diet as compared with animals fed the diet of Sherman and Merrill (1925) in which the concentrations of fats and proteins were lower than in the Osborne-Mendel diet. These rations are not only deficient in cystine, but also low in proteins. The latter value in the Osborne-Mendel ration is about 7·8 per cent. whereas that in the Sherman-Merrill diet only approximately 4·7 per cent. However, in addition to their low cystine and protein values these diets are also low in certain other constituents. For instance, the Osborne-Mendel diet is low in the vitamin B-complex whereas the Sherman-Merrill diet is low in mineral salts (except NaCl) and the vitamin B. However, these authors found that the addition of amounts of cystine adequate to produce good growth did not alter the toxicity of the sulphur. Nevertheless, even with the addition of cystine to the basal diets, the growth obtained was unsatisfactory and it is probable that the high toxicity of elementary sulphur, when incorporated in these diets, was due mainly to the lack of enough growth-promoting substances and not to that of any particular sulphur detoxifying agent. The experiments to be recorded in this paper were therefore carried out with the object of putting this supposition to the test.

EXPERIMENTAL.

Young white rats were used as experimental animals and they were kept under similar conditions as previously described (Kellermann, 1938, 2). The animals were weighed once weekly. They were given an excess of food and the daily consumption per rat recorded The composition of the rations is given in Table I.

TABLE I.

Percentage of Rations in Percentage by Weight.

	Normal diet.		Diet low in vitamins A and D.	Diet low in vitamin B-complex.	Diet low on vitamins.		Diet low in most minerals.		Diet low in proteins.		Diet low in proteins and low in most minerals.		Diet low in proteins, low on most minerals and low in vitamins.	
Designation of diet	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.	N.
"Vitaminized" starch	10	10	10	—	—	—	10	10	10	10	10	10	—	—
Dextrinized starch	39·4	34	34	44	49·4	44	45	39·6	53·5	48·1	59·1	53·7	69·1	63·7
Casein	20	20	20	20	20	20	20	20	5·6	5·6	5·6	5·6	5·6	5·6
Sucrose	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Salt 40	4·5	4·5	4·5	4·5	4·5	4·5	1·0	1	4·5	4·5	1	1	1	1
Lard	9·6	12	14	12	11·6	14	7·1	9·5	9·6	12	7·1	9·5	9·1	11·5
Agar	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5
Cod-liver oil	2	2	—	2	—	—	2	2	2	2	2	2	—	—
NaCl	—	—	—	—	—	—	·4	·4	—	—	·4	·4	·4	·4
l-cystine	—	—	—	—	—	—	—	—	·3	·3	·3	·3	·3	·3
Sulphur	—	3	3	3	—	3	—	3	—	3	—	3	—	3

All fourteen rations contained isodynamic quantities of energy* and the vitaminized starch " and salt 40 were prepared as described previously (Kellermann, 1938, 2). It was deemed advisable to make the rations low but not necessarily deficient in the various essential constituents because, although 3 per cent. of elementary sulphur had little effect on rats receiving normal diets, Lewis and Lewis obtained very detrimental results with rats on an inadequate diet (Sherman-Merrill) containing only 1 per cent. of flowers of sulphur. After the rats had been on the various diets for six weeks, they were killed by a blow on the head and certain internal organs immediately removed and preserved in a formalin solution for histopathological examination.

From the growth and food consumption records given in Table II it is evident that the incorporation of sulphur in rations, no matter whether they were physiologically complete or not, reduced food consumption and subsequent growth. In the case of the complete ration the addition of sulphur was of little effect inasmuch as the sulphur fed rats consumed on an average only 0·5 g. less food per day and gained 6 g. less over a period of six weeks with hardly any difference in the percentage gains. On a diet low in vitamins the animals still gained 13·3 per cent. but when sulphur was added to the same diet they lost 21·9 per cent. of their original weight. Similarly, when only the vitamin B-complex was low, the addition of sulphur resulted in a loss of 24·6 per cent. but, on the other hand, still gained 37·9 per cent. when the diet was lacking in vitamins A and D. This may be expected in view of the fact that the B-vitamins are water soluble, consequently their deposits in the body are sooner depleted, and their absence, therefore, sooner made to bear on the organism (Osborne and Mendel, 1919), than those of the fat soluble vitamins (Robertson, 1916). Likewise, on the average the rats gained on all the remaining incomplete rations but, with the exception of the low mineral diet, lost considerably when sulphur was added. The low mineral diet contained 1·98 per cent of ash (ashed at 450-500° C.), and it seems as if this concentration of ash was sufficient to support a fair amount of growth even though the diet contained 3 per cent. of elementary sulphur. However, when the difference in body gain of the animals on the control and sulphur low mineral rations is compared with the difference in gain and loss of the rats on the corresponding low protein and low vitamin rations, it is seen that the addition of sulphur to the low mineral diet resulted in a difference in gains of 66·7 per cent. whereas in the case of the low protein and low vitamin diets the differences were 67·5 and 35·2 per cent. respectively. This shows that not only proteins and vitamins, but also the essential minerals play an important rôle in counteracting the deleterious effects of elementary sulphur on growth. A multiplication of the defects, by limiting the proteins, vitamins and minerals, in one and the same diet, did not alter materially the final position in so far as change in weight was concerned.

In order to see whether the reduction in growth was due solely to a restriction of food consumption or whether it was accompanied

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* The energy values of the diets were calculated as described previously (Kellermann, 1938, 2).

TABLE II.

Summary of Growth and Food Consumption of Rats on various incomplete Rations with and without the addition of 3 per cent. Elementary Sulphur.

Ration.	Supplemented with.	Number of animals.	Initial weight. g.	Gain + or loss − g.	Percentage gain + or loss −	Total food. g.	Food per rat per day. g.
Normal	–	6*	63	+ 77	+ 122·2	300	7·1
Normal	Sulphur	6	57	+ 71	+ 124·5	277	6·6
Low in vitamins A and D	Sulphur	6	66	+ 25	+ 37·9	220	5·2
Low in vitamin B-complex	Sulphur	6	65	– 16	– 24·6	137	3·3
Low in vitamins	–	6	60	+ 8	+ 13·3	177	4·2
Low in vitamins	Sulphur	6	73	– 16	– 21·9	155	3·7
Low in most minerals	–	6	68	+ 63	+ 92·6	333	7·9
Low in most minerals	Sulphur	6	77	+ 20	+ 25·9	191	4·5
Low in proteins	–	6	76	+ 35	+ 46·0	292	6·9
Low in proteins	Sulphur	6	79	– 17	– 21·5	176	4·2
Low in proteins and low in most minerals	–	6	77	+ 25	+ 32·5	284	6·8
Low in proteins and low in most minerals	Sulphur	6	75	– 25	– 33·3	161	3·8
Low in proteins, low in most minerals and low in vitamins	–	6	74	+ 7	+ 9·4	239	5·7
Low in proteins, low in most minerals and low in vitamins	Sulphur	6	73	– 24	– 32·9	151	3·6

* From the time that an animal dropped out of a group as the result of death (see graphs, Fig. I) the results represent the average of the remaining animals.

by a breakdown of tissues due to sulphur toxicosis, the average body weights and food consumption per 100 g. body weight were plotted against the days of the experiment. The results are depicted graphically in Fig. 1.

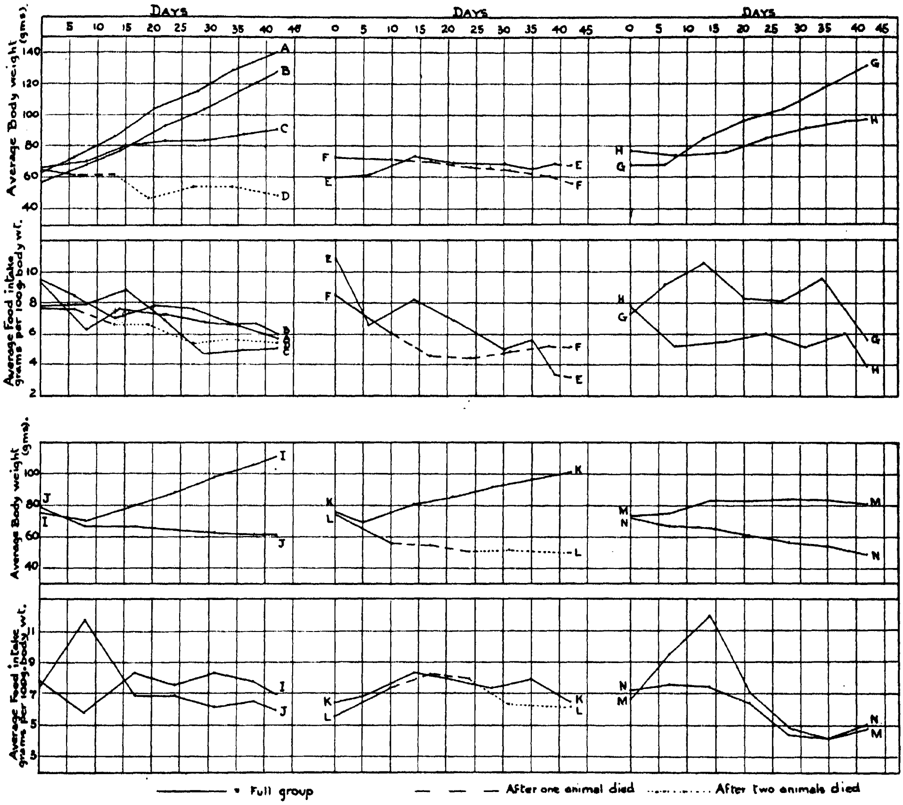

Fig I

Average body weight and food consumption of rats on a normal and various incomplete rations with, and without, the addition of 3% elementary sulphur.

The graph shows that the addition of elementary sulphur to the various diets invariably reduced the growth rate. In most of the incomplete rations the incorporation of sulphur actually resulted in a loss of weight. From the graphs giving the food consumption per 100 g. body weight it is evident that, with the exception of the low mineral diet plus sulphur (graph H), the inhibition of growth was not due to a lowered food intake per unit of body weight. As might be expected there was a gradual drop in the food intake per 100 g. body weight as the animals grew older [Macallum (1919), and Levine and Smith (1927)]. The deleterious effects of sulphur on growth should then mainly be ascribed to the reduced food intake per rat per day as shown in the last columns of Table II. This observation substantiates the results of Franke (1934) and Franke and Potter (1934) who showed that the poor growth of animals on a toxic diet was mainly due to a restriction of food intake.

The histological examination of the livers showed that, on the whole, the degenerative changes varied not only in degree and distribution in the various groups, with and without the administration of sulphur, but also in animals fed the same diet. These changes consisted of cloudy swellings and vacuolation in groups of hepatic cells situated at the periphery of some lobules. Furthermore, in a number of cells, the protoplasm had a tendency to disintegrate and to droplet (hyalin) formation. In addition to the above changes observed in the livers of rats that were killed at the conclusion of the experiment, the livers of animals that died showed haemorrhagic necrotic areas with slight or more advanced interstitial hepatitis.

DISCUSSION.

Whether the hepatic necrosis was the ultimate cause of death is difficult to say. However, inasmuch as no bacteria were seen in these lesions together with the fact that all the animals that succumbed, received elementary sulphur in their food, seem to show that the animals died from sulphur toxicosis. These results therefore substantiate the observation of Lewis and Lewis (1927) that the ingestion of appreciable amounts of elementary sulphur by rats can exert toxic and even fatal effects on the animals. However, this statement should be accepted with some reservation because, in the writer's experience, elementary sulphur will have definitely toxic effects only under certain conditions. One of these conditions is that the basal ration should be deficient in one or more respects so as to retard or stop growth. As already pointed out in Table II, one of the main effects of sulphur was to reduce food consumption and the subsequent gain in weight. As a matter of fact in some cases the addition of sulphur resulted in a loss of 30 per cent. or more of the animals' initial weight, and it would seem, therefore, that under the experimental conditions, the greater the inhibition of growth the more susceptible the animals become to sulphur toxicosis.

If this viewpoint is correct it will help to explain why the animals of Lewis and Lewis were more resistant to the toxic effects of sulphur on the Osborne-Mendel diet than on the Sherman-Merrill (1925) one in view of the fact that the former diet is the better balanced of the two being richer in proteins, minerals and vitamins A and D. However, Lewis and Lewis suggested that the high fat content of the Osborne and Mendel diet might be partly responsible for the lessened toxicity as " the fat might form a coating around the particles of sulphur and thus make more difficult the intimate contact of the sulphur with the intestinal mucosa or the action of bacteria." This explanation seems to be untenable as the writer did not find (unpublished data) any difference in the absorption of elementary sulphur on diets low and high in fat content. Furthermore, young rats fed these respective diets, containing 3 per cent. of elementary sulphur, over a period of eight weeks, did not reveal any difference in their general well-being (Kellermann, 1938, 2).

Another factor would seem to be the method of sulphur administration. Swart (1936) expressed the opinion that the feeding of sulphur mixed with the feeds might be less toxic than when it is dosed as he fed his animals 9·5 g. of flowers of sulphur per sheep per day over a period of 10 months without any ill-effects, whereas Steyn (1931) observed that the dosing of an appreciably smaller amount of flowers of sulphur, namely 45 g. per sheep per week, over a period of thirty-five days caused symptoms of poisoning and high mortality. Therefore, it would be of interest to investigate this problem further, especially with the view of ascertaining whether rabbits and guinea-pigs are actually as susceptible to sulphur poisoning as found by Lawson, Redfield and Boyd (1934) whose animals all died within twenty-four hours after receiving doses of from 0·5 to 2·0 g. of either colloidal or flowers of sulphur per Kg. of body weight into the stomach through a small stomach tube.

SUMMARY.

1. Experiments are described in which were studied the effects of incomplete rations on the toxicity of elementary sulphur.

2. The incorporation of sulphur in the diet exerted toxic effects only when the basal ration itself could not support normal growth as the result of one or more deficiencies. The chief effect of sulphur under such conditions was to reduce food consumption per animal but not per unit body weight.

3. It would seem that vitamins, proteins and minerals were all equally important in counteracting the deleterious effects of the sulphur.

4. Although the rats lost considerably in weight when sulphur was added to the defective diets, the livers of the animals that were killed at the conclusion of the experiment did not show any constant pathological change whereas the same organs of those animals that died during the experiment exhibited haemorrhagic necrotic areas with slight or more advanced interstitial hepatitis.

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Section IV.

Nutrition.

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The Endogenous Nitrogen Metabolism of Sheep with Special Reference to the Maintenance Requirement of Protein.

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SINCE the classic work of Folin in 1905, in which he differentiated between an exogenous and an endogenous form of protein catabolism, much work has appeared to test the validity of his postulation. While there exists a general agreement on the exogenous form of protein catabolism, the literature is somewhat divided on the nature and biological importance of the endogenous form of protein metabolism. Folin regarded the latter form of catabolism as a constant type of catabolism in comparison with the more variable excretion of nitrogenous end-products arising from exogenous origin. Osborne and Mendel (1914) as well as Sherman (1920) question the constancy of the endogenous nitrogen and claim that if a completely efficient protein is administered, the endogenous nitrogen would be minimized. Mitchell (1930), however, who investigated this point very carefully by supplying small quantities of protein of a high biological value, could not find any evidence in support of the theory of the above workers, but agrees with Folin, that the endogenous nitrogen is a biological constant. Smuts (1935) found a definite relationship between the endogenous nitrogen metabolism and the basal metabolism of different species of animals varying widely in weight. Since the latter measurement is fully recognized as being reproducible and constant for an individual, it follows that the endogenous nitrogen is a measure of equal constancy.

Furthermore the preponderance of evidence in the literature definitely favours a constant output of endogenous nitrogen, which can be related either to body weight or surface area.

While extensive work has been conducted on the endogenous nitrogen metabolism of rats and other types of omnivora, scanty attention has been given to this type of metabolism in ruminants.

This fact is mainly accounted for by the extreme difficulties encountered in the computation of suitable nitrogen-free rations, which are palatable and easily consumed. Nevertheless Morgin (1912) and his co-workers, Satola (1930), and Turk and his collaborators (1934), succeeded to induce sheep to eat a low nitrogen ration consisting mainly of straw, starch, sugar, oil and minerals. In our study on the determination of the biological values of feeds for larger farm animals, it became essential to compound a suitable nitrogen-free ration for sheep. This has been successfully executed and the results reported in this text.

EXPERIMENTAL.

Mature merino wethers were used throughout this study. The best eaters were selected and were then put on an exclusive lucerne ration for a month. After this, they were directly transferred to the metabolism cages of the Forbes type and put on a nitrogen-free ration. Quantitative collection of the urine in 2 per cent. HCl was carried out daily and analysed for total nitrogen (Kjeldahl method).

Creatinine (creatinine and creatine), total sulphur, and neutral sulphur were determined. The creatinine was determined according to the microchemical modification of Folin's method as reported by Folin (1924). The total sulphur was determined by method of Denis (1910). Gas flames were avoided in all evaporating processes. The faeces were collected daily, stored in airtight jars, and aliquots analysed for total Nitrogen at the end of the collection period. Sheep were fed once daily.

The composition of the nitrogen free ration is given in Table 1. The final composition as reported is the outcome of several attempts in which certain ingredients were omitted and others added. It was found that the inclusion of sugar, even at as low a level as 5 per cent., invariably caused looseness of the bowels. The addition of different types of oils was tested out. It was found that they either did not mix well with the ration or put the sheep completely off feed. Ordinary maize starch was found to be too pulverized with the result that dextrinized starch was resorted to. This proved very successful. The dextrinized starch was ground into small pieces equivalent in size to the maize kernel. These the sheep consumed excellently and some even preferred it to maize. When putting sheep on the nitrogen-free ration, it was found advisable to start them off with 150 to 200 grams of the mixture and increase the daily feed until they just cleared up their daily allotment. With this ration under our conditions, we did not experience any difficulty with feed refusal or reduction in weight.

TABLE I.

Composition of Ration.

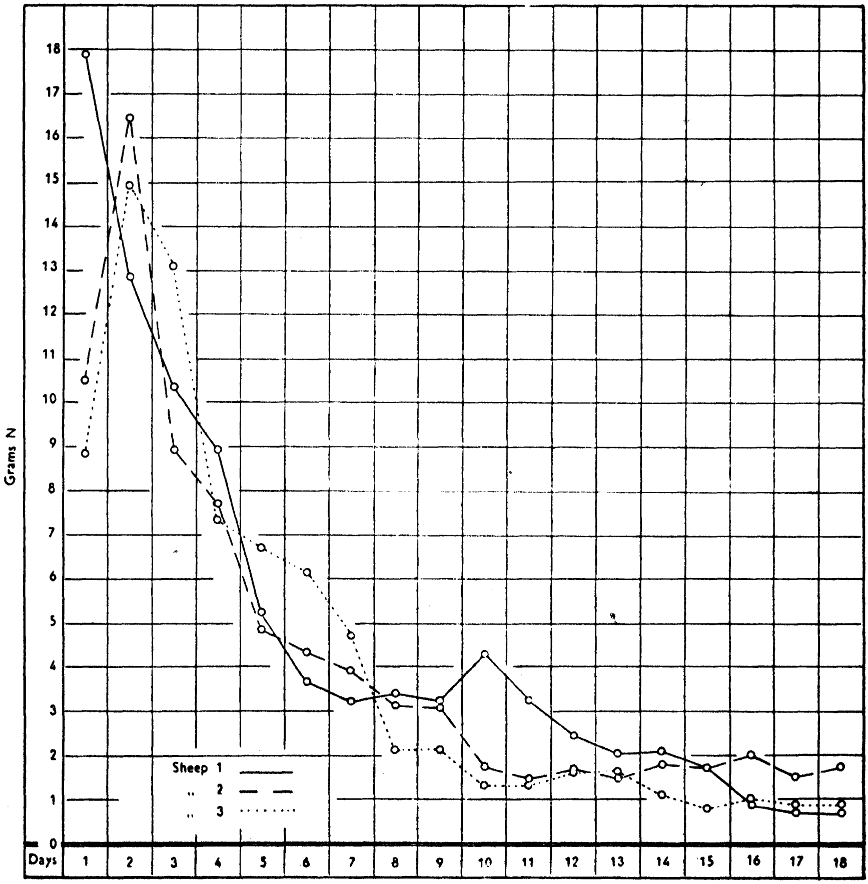
Dextrinized starch	73·0
Agar	20·0
Cod liver oil	2·0
Bone ash(¹)	3·0
Salt	2·0
TOTAL	100·0

(¹) Bone ash contains 42·5 per cent. Ca and 17·04 per cent. P.

Experimental Results.

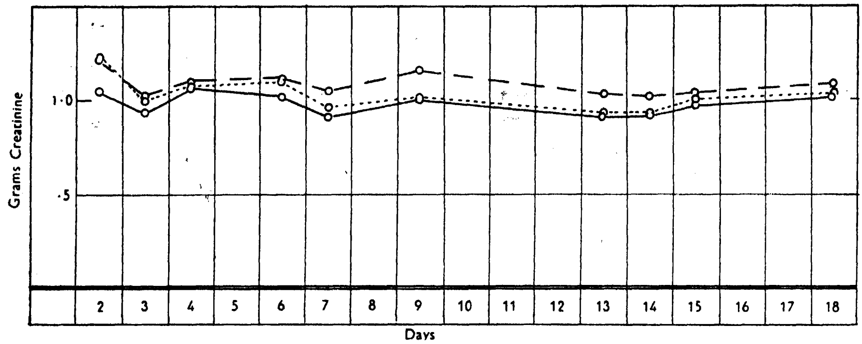
Three of the sheep which had been kept for a month on an exclusive lucerne ration of 14 per cent. protein, were put on the nitrogen-free ration and urine collections made daily. The curves representing the total daily nitrogen excretion are shown in graph 1. The low figures obtained with sheep 2 and 3 for the first day are undoubtedly due to the small quantities of urine voided, probably as a result of the change in quarters. It is apparent from the graphs that there is a sharp decline in total nitrogen excretion from the second day to approximately the eighth day. From then onwards the decline in the curve is considerably reduced. From these results it appears as if sheep 3 has attained its endogenous level round about the fourteenth day and sheep 2 at the eleventh day, while sheep 1 only reached a steady output of nitrogen from the sixteenth to the seventeenth day. Sheep 1 was the best eater of the three, which fact may lead one to suspect that the nutritional condition may be a factor in determining the length of the Nitrogen low period before the endogenous level is actually attained.

Graph 1.

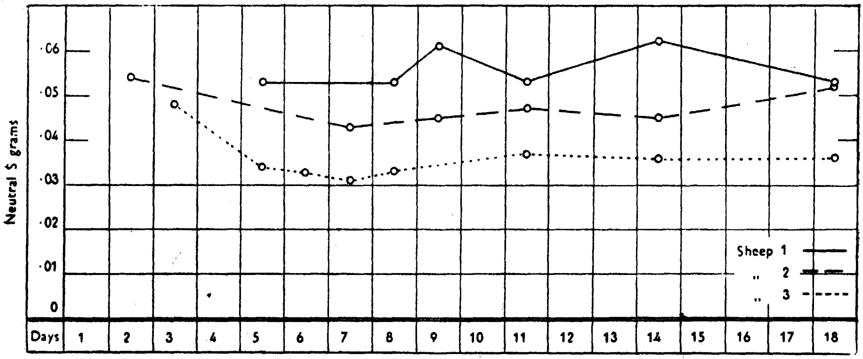


In graph 2 the daily excretion of creatinine for each sheep is given. It is evident that the excretion of this nitrogenous end-product is not influenced by the distinct decrease in total nitrogen during the first eight days. There is a tendency with all three sheep to show a slightly elevated excretion of creatinine during the first two days, probably as a result of preformed creatine in the previous ration. From the third day, however, the excretion is very uniform for all three sheep and almost assumes a straight line. The neutral sulphur is very constant for sheep 2 and 3 but varies somewhat in sheep 1. The elevated points on the curve of sheep 1 at the ninth and the fourteenth day are unexpected, since the other four determinations are absolutely of the same magnitude.

GRAPH 2.

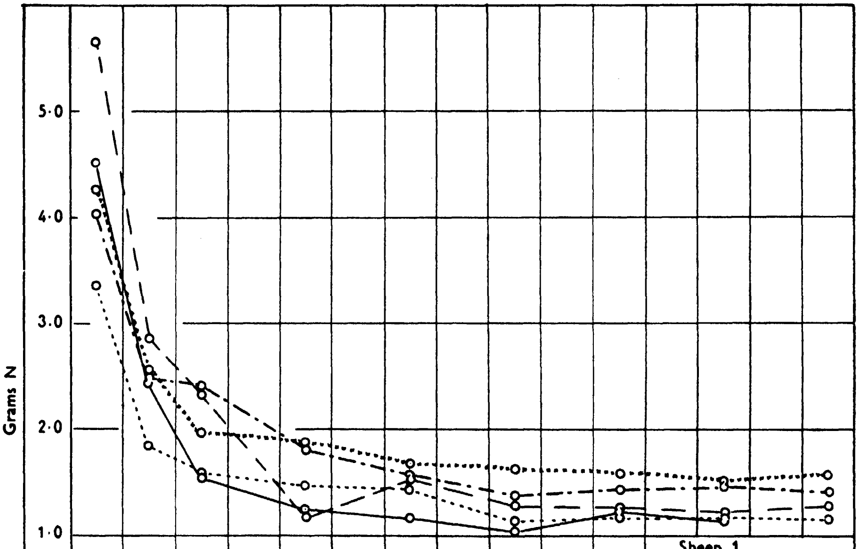


GRAPH 3.



In graph 4 are reproduced the results of four sheep which were kept for three months on an 8 per cent. lucerne protein ration supplemented by starch. The initial sharp fall in the daily nitrogen excretion occurred in this case during the first two days, while in the case of the 14 per cent. protein, it stretched over the first eight days. The endogenous level on the average for the four sheep seemed to be obtained after the sixth day. These results therefore seem to indicate that the deposit protein stores were decreased during the period of lower level protein feeding. At the 8 per cent. level of protein feeding, the period to reduce these sheep to their endogenous level was almost half that necessary to attain a constant excretion of nitrogen on a 14 per cent. protein level. This point strongly emphasizes the necessity of employing a standardized protein ration, before the endogenous nitrogen excretion is measured. It can readily be appreciated how a difference in level of protein may cause a difference in the ultimate endogenous N figures, due to the fact that the actual endogenous level is not attained. It seems, that this point may not only influence the reproducibility of the biological values of feeds, but also account for the lower endogenous Nitrogen figures at the end of a series of experiments, as suggested by Ashworth (1935). However, Ashworth's explanation that the lowered endogenous Nitrogen figures are caused by a reduced reserve protein due to continued Nitrogen-free feeding, is not supported by our findings. It is of course recognized, that just as the basal metabolism may be reduced through prolonged captivity so may the endogenous N be influenced. But under ordinary conditions of determining the biological value of feeds, the period of confinement does not seem to be long enough to effect such a reduction.

Graph 4.



Four sheep were taken off a lucerne ration and transferred to a Nitrogen-free ration, on which they were kept for 20 days. They were then put on an 8 per cent. lucerne protein ration for 20 days, on a similar ration supplemented by cystine for the same period and ultimately for 25 days on a nitrogen-free ration. The endogenous nitrogen per Kg. weight for the four sheep was as follows: ·036, ·048, ·035 and ·033 grm. respectively at the beginning of the experiment and ·033, ·040, ·033 and ·033 grm. at the termination of the experiment. These results certainly support the constancy of the endogenous nitrogen metabolism. The lowered endogenous nitrogen noted by Ashworth (1935) is therefore in all probability due to the fact that the endogenous level was in reality not attained at the start of the experiment on account of too short a preliminary N free period, the length of which, as has been shown, being in a great measure determined by the level of protein intake.

Our average figure of ·035 grm. endogenous N per Kg. weight agrees very well with that reported by Satola but is much lower than the value of ·063 reported by Hutchinson and Morris (1936).

In Table 2 is given the distribution of the nitrogenous and sulphur end-products in the urine of sheep kept on a nitrogen free ration. The creatinine nitrogen constitutes a larger portion of the total nitrogen as the sheep reached the endogenous level. During the first day or two after the sheep have been taken off the protein ration, the creatinine only makes up 2 per cent. of the total nitrogen excretion. When these animals have attained their endogenous level the creatinine as shown in Table 2 makes up approximately 37 per cent. of the total nitrogen excretion. The diminished nitrogen excretion is therefore in greater part due to the cessation of exogenous catabolism of protein, and consequently to a decreased excretion of urea which is the chief end-product of exogenous protein catabolism. Smuts (1935) has shown that as the size of the animal increases, the creatinine assumes a larger proportion of the endogenous nitrogen. In mice it is approximately 5 per cent., in guinea pigs 7 per cent., in rabbits 14·3 per cent. and in pigs 14·3 per cent.

TABLE II.

Distribution of Urinary end-products of Sheep on N-free Ration.

Animal No.	Weight Kgrms.	Endog. N Grm.	Creatinine N Grm.	Creatinine coefficient.	Creatinine N Per cent. of End N.	Total S. Grm.	Neutral S. Grm.	Ratio N/S.
1	33	1·10	0·41	12	44	0·129	0·055	8·5
2	35	1·40	0·41	12	29	0·203	0·056	6·9
3	36	1·29	0·48	13	37	0·216	0·069	6·0
5	42	1·19	0·44	11	37	0·145	0·039	8·2
Average	—	—	—	12	37	—	—	7·4

The creatinine coefficient expressed in terms of Schaefer's definition, namely, milligrams creatinine nitrogen per Kg. weight, is recognized by physiologists as an index of muscular mass and development. For this reason it is not a very uncommon phenomenon to find a variation in this index, depending on the fitness of an animal. The creatinine coefficient, obtained for sheep in this study, namely 12, falls in line with the figures reported for man and pig. For smaller animals like the mouse, rabbit and guinea pig the creatinine coefficient is larger. For guinea pigs this figure as reported by Smuts (1935) is approximately 14, for mice 30 and for rabbits 17.

The ratio of sulphur to total nitrogen excretion is often taken as an index of the type of catabolism which is proceeding in the body. Under fasting conditions where tissue catabolism takes place this ratio is approximately 16. In as much as our figure of 7·4 is much lower, it affords additional evidence that our animals were strictly on a non-tissue catabolizing level.

The maintenance requirement of an animal for protein seems to be determined by the inevitable and constant loss of essential nitrogenous constituents of the tissues as a result of the endogenous catabolism. Consequently there is a certain amount of food protein or other available sources of nitrogen needed to replenish this loss of nitrogen in order to maintain the normal functioning of tissues and the life of the animal. This constant basal need of protein is comparable to the basal needs of energy, and forms the foundation on which are superimposed the protein requirements for growth, lactation and other functions.

From the above reasoning it is evident that the maintenance requirement of nitrogen of an animal may, therefore, be measured by the total nitrogen excretion in the urine of an animal kept for a long enough period on a nitrogen free ration to establish its endogenous catabolism. For the four sheep under investigation an average figure of ·035 grms. nitrogen per Kg. weight was obtained. However, in later determinations on 10 sheep, it was found that values for the endogenous nitrogen vary from ·035 grms. to ·048 grms. per Kg. weight, with an average value of ·041. Taking the latter figure as more representative, it would mean that the protein required for maintenance per Kg. weight is equivalent to ·256 grms. This value naturally represents protein actually utilized by the body. Therefore to convert it to digestible crude protein, the biological value should be taken into account. If, as in the case of lucerne, the biological value for sheep is 60, then the digestible protein requirements become $\cdot 256 \times \frac{100}{60} = \cdot 43$ grms., which is equivalent to 19 grms. digestible protein per 100 lb. weight. The corresponding amount of dietary protein would be larger than this, and would be greater the greater its wastage in metabolism and hence the smaller its biological value. Consequently, when a protein feed with a lower biological value than that of lucerne is utilized, a proportionately larger amount of digestible protein is necessary. However, if we allow a fair margin of safety and assume an average biological value of 50 for maintenance, then the maintenance requirement in terms of digestible protein for a 100 lb. sheep becomes 23 gms. This figure is somewhat lower than 27 grms. digestible protein, proposed by Armsby (1917) and almost three times lower than that proposed by Henry and Morrison (1923).

Smuts (1935) adapted the Brody equation $Q = 70{\cdot}4m^{\cdot 734}$ for the prediction of the basal metabolism of mature mammals to a determination of the protein requirements by the use of his ratio 12·5 mgm. protein per calorie of basal heat.

The equation then became $P = {\cdot}88\ M^{\cdot 734}$, where P is the day's requirement of protein expressed in grams. The protein requirements obtained in this way do not refer to dietary protein or digestible protein but to protein actually utilized. Assuming therefore a biological value of 50, the digestible protein requirements per 100 lbs. live weight for sheep become 29 grams. This predicted figure compares favourably with the determined value of 23 grams digestible protein. It appears, therefore, that this equation can be applied successfully to the determination of the maintenance protein requirement of sheep. On the other hand, by reversing the calculation, the basal energy needs of sheep may be predicted with equal ease. Hence 29 grms. digestible protein having a biological value of 50, is equivalent to 14·5 grms. utilizable protein. By applying the Smuts equation for the relationship between endogenous nitrogen and basal metabolism, namely 12·5 mgm. P = 1 Calorie basal heat, a figure of 1160 Calorie basal heat per 100 lb. sheep is obtained. This figure agrees well with the reported data on the basal metabolism of sheep. Ritzman and Benedict (1930) report a value of 1260 Calories per 100 lb. sheep. This figure was obtained by deducting 17 per cent. from their standard metabolism, and may therefore be slightly high. Rubner's Standard of approximately 1040 Calorie is slightly below our predicted value, while Krog's standard of 1248 Calories per 100 lbs. is slightly higher. However, the differences are so small that the relationship may be successfully utilized in predicting the basal energy needs of sheep.

SUMMARY AND CONCLUSIONS.

By means of a nitrogen free diet the time period for the attainment of the endogenous nitrogen level has been established in sheep. It appears that the preliminary protein period is a determining factor in the length of the nitrogen free period. Sheep on a 14 per cent. protein were reduced to endogenous level in 14 to 15 days, while sheep kept on an 8 per cent. protein attain the endogenous level from the sixth to the eighth day of nitrogen-free feeding.

The partition of the urinary end products on a nitrogen free ration is discussed and special consideration is given to the maintenance requirement of sheep. It has been shown in this study, that the maintenance requirement of protein of a 100 lb. sheep is less than advocated in the usual standards.

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The Basal Metabolism of 3 to 4 weeks old White Leghorn Chickens.

By D. B. SMUTS and J. S. C. MARAIS, Section of Nutrition, Onderstepoort.

ARMSBY (1917), who introduced the conception of nett energy values, clearly discriminated between the energy values of feeds and the energy required by the animal. The latter requirement, he analyzed into definite and distinct physiological functions, such as basal metabolism, elaboration of new tissues, fat deposition, egg production, lactation, gestation, and work. The basal metabolism of an animal, which is its minimum energy expenditure compatible with the mere existence of life, is a necessary and essential step in the determination of the different components of an animal's total energy requirement. It is that fraction of the energy needs, on which the energy required for all the other fractions, such as growth, egg production, etc., are superimposed. A growing chicken would therefore first utilize its feed energy to cover its basal heat requirements, and thereafter convert the remaining energy for anabolic purposes such as tissue synthesis, fat production, etc. It is therefore self-evident, that in a study of the energy requirements of animals, the basal heat production should constitute the initial determination. Such a determination is generally conducted when the animal is in a post-absorptive condition and free from the stimulus of food, with an environmental temperature corresponding to the critical temperature of the animal. The experimental animal should be inactive and as nearly as possible at complete rest.

Under these conditions the basal metabolism of an animal or individual is readily reproducible, and appears to be one of the most constant of biological measurements. For this reason, it is also extensively used as a diagnostic measure for certain types of diseases. However, it has been amply demonstrated that the constancy of the basal heat production holds better when expressed per unit of surface. Consequently the surface area is used in preference to weight, for expressing the basal metabolism, since in the latter case differences in size of the animal are reflected in the more variable set of data obtained. As a result, formulae have been evolved by time consuming devices, to estimate the surface area of different species of animals for expressing the basal heat production.

While the basal heat production, generally speaking, may be regarded as a biological constant for mature animals, it is nevertheless definitely influenced by such factors as age and sex. Du Bois (1916) in his curve on the basal metabolism of humans, per unit of body surface, shows a striking change from birth to old age. The first weeks of life were marked by an extremely low level of metabolism approximating 27 to 30 calories per square meter per hour. From then onwards it rose rapidly until at one year of age, the metabolism is 50 per cent. above adult level. It appears that the maximum rate of metabolism occurring from 2-6 years of age is about 60 calories per square meter per hour. After this stage, the metabolism drops comparatively rapidly to 20 years of age and then very gradually afterwards. Mitchell and Carmann (1922) working on rats, showed, that the basal heat production at 30-40 days of age was distinctly higher than at 90-190 days of age. The same effect of age on the basal metabolism was noted by Deighton (1923) and Wood (1926) in growing pigs. Mitchell, Card and Haines (1927) found a remarkable similarity between the basal metabolism of chickens and humans, in that the metabolism per unit of surface is distinctly below the adult level at hatching, rising rapidly to a maximum and then decreasing to the adult level, which is maintained for a considerable fraction of the life span.

That the environmental temperature at which the basal metabolism determinations are conducted, influences the heat production values considerably, has been significantly demonstrated in studies on the respiratory exchange of animals. Within the range of thermal neutrality the heat production is unaffected. If, however, the environmental temperature becomes lower than the critical temperature of the animal, heat production is increased to cope with this extra demand for heat. It is therefore essential that the environmental or experimental temperature should coincide with the critical temperature of the animal under experimentation. Mitchell (1927) found the critical temperature for mature birds to be in the neighbourhood of 63° F. Kleiber (1933), in a study on the influence of environmental temperature on the utilization of food energy by baby chicks found the lowest CO_2 production at 40° C. From these data it would appear that the critical temperature of one-month-old chickens would lie approximately between 30° C. and 35° C. In this study the temperature has been maintained as far as possible about 31° C.

EXPERIMENTAL RESULTS.

1. *Surface area determination.*

Owing to the fact that no formula exists for chickens by which the surface area could be estimated, it was decided to utilize the existing material to formulate a prediction formula for the estimation of the surface area. After the basal heat production had been measured, the chickens were subjected to a period of fasting equivalent to that employed in the actual determinations, namely 18 hours. They were weighed, killed, de-feathered and the clean carcass washed with alcohol and ether. The Mold method originally used by Lee (1929) and successfully utilized by Smuts (1935) on mice and guinea-pigs, was then applied to the completely dry carcasses of the treated chickens. Each chicken was kept in a definite position while the gauze cemented with collodium ether was wrapped around it. The chickens were then left for some time in front of an electric fan for the mold to get thoroughly dry. The mold was then cut loose from the carcass and further sub-divided into as many small pieces as were necessary for tracing the areas on paper. These areas were subsequently determined by means of a planimeter and recorded as the measured surface area.

The feet were not included in the mold but cut off and traced separately. The area of each foot was multiplied by 2. This area was then added to the mold area and constituted the radiating area of the chicken.

The complete data of the measured surface areas and the corresponding equations with the percentage deviations are recorded in Table I.

TABLE I.

Data and Calculation of the Surface Area of White Leghorn Chickens.

Animal No.	Weight. Grms.	Measured Surface Area. Sq. cm.	$K = \frac{S}{W^{\frac{2}{3}}}$	Area according to $S = 11 \cdot 2W^{\frac{2}{3}}$ Sq. cm.	Percentage Deviation from measured Area.	$S = 36 \cdot 31$ W^{457} Sq. cm.	Percentage Deviation from measured Area.
624	254	471·9	11·8	449·2	— 4·81	445·5	— 5·6
285	235	434·3	11·4	426·5	— 1·80	440	+ 1·38
323	340	427·1	11·1	432·5	+ 1·26	444·3	+ 4·03
385	247	433·3	11·0	440·9	+ 1·75	450·2	+ 3·9
630	280	482·3	11·3	479·4	— 0·6	476·7	— 1·16
242	255	476·5	11·9	450·3	— 5·50	456·7	— 4·16
618	274	465·8	11·0	472·5	+ 1·44	472·1	+ 1·35
641	325	454·5	11·9	426·5	— 6·16	440·1	— 3·17
417	327	512·9	10·8	531·5	+ 3·65	510·6	— 0·45
628	292	495·0	11·3	492·9	— 0·42	486·1	— 1·8
602	239	452·4	11·7	431·3	— 4·66	443·5	— 1·97
293	339	493·3	10·2	544·5	+ 10·38	520·4	+ 5·49
604	252	469·8	11·8	446·8	— 4·9	454·3	— 3·3
650	319	497·2	10·7	522·9	+ 5·17	506	+ 1·77
425	277	479·6	11·5	475·8	— 0·8	474·4	— 1·08
636	320	486·2	10·4	524·0	+ 7·56	506·6	+ 3·9
637	374	588·5	11·3	581·5	— 0·2	556·9	— 5·37
226	279	451·3	10·6	478·2	+ 5·96	476	+ 5·47
Average	—	—	11·2—	—	3·77	—	3·06

By substituting the measured surface area and the weight of each bird in the Meeh formula $S = KW^{\frac{2}{3}}$, K the constant was determined for 17 individual chickens, and gave an average of 11·2. Using this value for K, and applying it to the above equation, the surface area was calculated from the individual weights and compared with the measured areas. As will be seen from Table I, column 2, a remarkably close fit was obtained. In only one case was the percentage deviation more than 10 per cent. Disregarding the signs the average deviation is 3·77 per cent. This value compares extremely well with deviations obtained with other small animals under similar conditions.

If, however, the exponent of W (weight in grams) in the Meeh formula as well as its constant (K) are evaluated from the above data by the method of least squares by using the following equations:—

$$(1)\ \Sigma \log S = N \log K + \quad \Sigma \log W$$

$$(2)\ \Sigma \log S.\ \log W = \log K. \quad \Sigma \log W + \quad S(\log W)^2$$

then even a better fit is obtained. The surface area predicted from the determined equation $S = 36{\cdot}31\ W^{\cdot 157}$ is given in Table I together with the percentage deviation from the measured areas. In no case was the percentage deviation greater than 6 per cent. The average percentage deviation disregarding signs is only 3·06. From these results it would appear that the latter formula can be applied with success in predicting the surface area of approximately one month old chickens.

2. *Basal metabolism.*

In studying the effect of length of fasting on the respiratory exchange of chickens the Haldane Gravimetric method as modified by Mitchell (1926) and previously employed by one of the authors was used in this study. The outgoing chain consisted of two rows of bottles which were connected to the animal chamber by a two-way stopcock. In case of abnormal activity the current of outgoing air could be diverted to the one train, the other train absorbing only when the animal was quiet. The latter train consisted of small bottles which could be accurately weighed. The animal chamber consisted of a fruit jar into which the chicken fitted snugly but with apparent comfort during the period of observation.

Chickens were fasted for 12 and 18 hours respectively after which period they were subjected to a basal heat determination. The results of the 12-hour basal metabolism determinations are given in Table II. The heat production was computed from the CO_2 production by using Lusk's figures, on the assumption that the total respiratory quotient was non-protein. The usual errors resulting from this simplification as shown by Mitchell, are less than 2 per cent. The environmental temperatures varied from 29° C. to 35° C., with the majority of cases falling between 30° C. and 32° C. In some cases chickens were first subjected to an 18-hours fast and later to a 12-hours fast. This procedure was found necessary in order to avoid the beginning of a fast in the early morning before the animals had fed.

In studying the effect of fasting on the respiratory quotient and metabolic rate, it was originally intended to include a 24 hours fasting period, but due to the fact that the respiratory quotients attained after 18 hours were so strongly representative of a fasting R.Q. this idea was abandoned. As will be seen from Table II the R.Q.'s after 12 hours fasting are still well above the customary R.Q. of ·70 for an animal in the post-absorptive state. The individual R.Q.'s vary somewhat, but with the exception of a few low values, they all lie in the neighbourhood of ·80, giving an average of ·78. This would indicate that at this stage of fasting the animal has not reached a post-absorptive state but was still oxidizing a mixture of fat and protein. Consequently the average heat production of 1,060 calories per square meter could not be interpreted as the basal heat production, since portion of the heat produced may be due to the stimulus of food present in the alimentary canal.

TABLE II.

Heat Production of 3-4 *Weeks Old White Leghorn Chicks after* 12 *hours fasting.*

Chicken No.	Weight. Grms.	Surface area. Sq. cm.	R.Q.	O_2 per day. Liters.	HEAT PRODUCTION.			Average Temperature.
					Per 24 hours. Cal.	Per Kg. body weight. Cal.	Per Sq. m. of Surface. Cal.	
240	259	460·2	·79	9·74	46·64	180·1	1,013·5	32°C
242	234	439·2	·78	9·74	46·52	198·8	1,058·8	30°C
266	234	439·2	·74	9·74	46·04	196·8	1,048·3	32°C
285	118	321·2	·80	8·23	39·51	334·8	1,230·1	30°C
293	193	402·3	·78	8·57	40·93	212·1	1,017·4	30°C
323	109	309·8	·80	5·54	26·60	244·0	858·6	29°C
385	175	384·6	·71	9·07	42·54	243·1	1,106·1	32°C
417	285	480·6	·73	12·26	57·79	202·8	1,202·5	33°C
425	155	363·9	·73	9·74	45·91	296·2	1,261·6	32°C
602	203	411·6	·76	9·74	46·27	227·9	1,124·4	31°C
604	184	393·6	·82	7·56	36·48	198·4	926·8	32°C
624	140	347·3	·79	6·22	29·78	212·7	857·5	31°C
628	300	492·0	·84	11·42	55·39	184·6	1,125·8	32°C
618	198	407·0	·76	9·07	43·09	217·6	1,058·7	31°C
630	209	417·2	·81	8·23	39·61	189·5	949·4	32°C
641	217	425·3	·76	9·58	45·51	209·7	1,070·7	31°C
650	234	439·2	·83	10·08	48·77	208·4	1,110·9	32°C
Average	—	—	·78	—	—	221·0	1,060·1	—

In Table III the respiratory quotients and heat production per square meter after an 18 hours fast are given. The respiratory quotients approximate an average value of ·72. The heat production per square meter averages 891 calories, a value much lower than that obtained after 12 hours fasting. From these R.Q.'s it is evident that the animal is in a post absorptive state and oxidizing its own fat. In work of this nature an occasional R.Q. of less than ·70 is encountered to which no special significance is attached other than the oxidation of fat. Henry, Magee and Reid (1934), however, fasted a cock for 48 hours and obtained an R.Q. of ·69. This R.Q. was maintained over long periods of fasting. They maintain that such an R.Q. was not due to fat oxidation direct or indirect through conversion to sugar. They suggested that an R.Q. lower than ·70 may be associated with protein metabolism, on account of the fact that a large proportion of the nitrogenous end-products in the fowl are excreted as uric acid and not as urea. Their argument is partly based on the following equations:—

$$2CH_3{\cdot}CH\ NH_2COOH + 6O_2 = (NH_2)_2CO + 5CO_2 + 5H_2O$$

$$RQ = \frac{5\ CO_2}{6O_2} = {\cdot}838$$

$$8CH_3{\cdot}CHNH_2{\cdot}COOH + 21O_2 = 2C_5H_4N_4O_3 + 14CO_2 + 24H_2O.$$

$$RQ = \frac{14\ CO_2}{21O_2} = 0{\cdot}667$$

When urea is therefore the end-product of protein metabolism, an R.Q. of ·838 is expected, while in the case of the fowl where uric acid is the end-product, the R.Q. of protein metabolized will be ·667.

It is difficult to understand why an animal such as the fowl will metabolize protein in preference to fat in a fasting condition, if it has the latter nutrient available.

TABLE III.

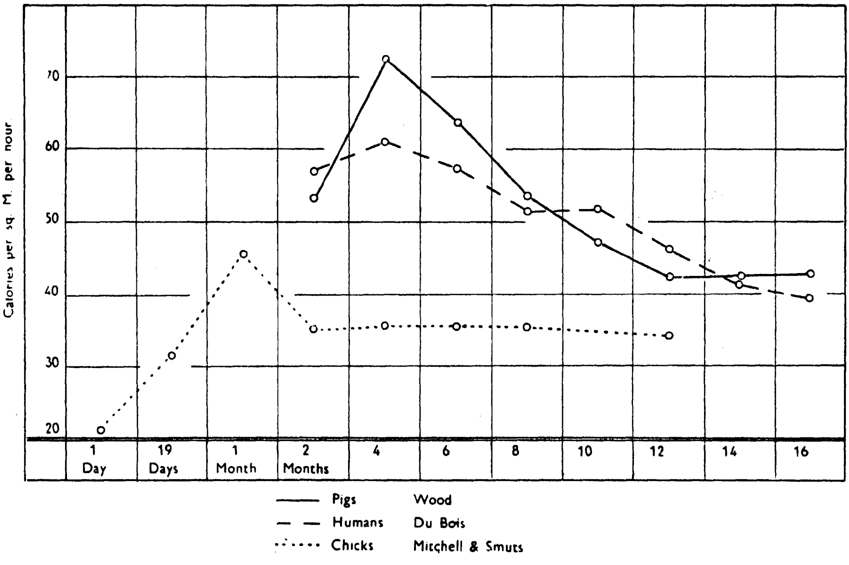
Heat Production of 3-4 *Weeks Old White Leghorn Chickens after* 18 *hours fasting.*

Chicken No.	Weight.	Surface Area.	R.Q.	O_2 per day.	Heat Production.			Average Temperature.	Heat Product per 24 hours per Sq. m. calculated from S = 81·9 W·705
					per 24 hours.	Per Kg. body weight.	Per Sq. m. of surface.		
	Grms.	Sq. cm.		Liters.	Cal.	Cal.	Cal.	°C	
240	217	425·3	·76	8·58	40·76	187·8	958·4	30	1,120
242	143	350·8	·74	6·22	29·40	205·6	838·1	32	1,085
266	234	439·2	·70	9·41	44·10	188·5	1,004·1	30	1,148
285	149	357·7	·77	6·05	28·82	193·4	806·1	31	1,033
293	198	407·0	·72	6·05	28·45	143·7	699·0	32	834
323	96	292·3	·70	5·71	26·76	278·8	915·5	29	1,305
385	124	328·6	·71	7·39	34·66	279·5	1,023·6	35	1,415
417	262	462·6	·74	10·75	50·82	194·0	1,098·6	30	1,219
425	255	456·8	·71	9·24	43·43	170·0	948·8	33	1,065
602	256	457·6	·72	9·07	42·65	166·6	932·0	32	1,021
604	233	438·4	·74	8·23	38·90	167·0	887·3	31	1,018
624	151	363·9	·70	5·38	25·21	167·0	692·8	31	894
628	217	425·3	·70	8·74	40·10	184·8	942·9	34	1,102
618	156	365·0	·71	7·06	33·11	212·2	907·1	30	1,150
630	269	468·1	·71	8·57	40·16	149·3	857·9	30	956
841	154	362·8	·70	5·88	27·55	178·9	759·4	33	967
650	160	369·3	·73	6·89	32·48	203·0	879·5	32	1,109
Average	....	......	·72	—	—	183·5	891·2	—	1,085

It is nevertheless a strange phenomenon that only occasional cases of R.Q.'s less than ·70, appear in a series of determinations. Mitchell (1927), in 58 determinations of basal metabolism after 48 hours fast only obtained five lower than ·70. Probably the nutritional state of the bird may be an influencing factor.

From our data it appears that chickens of one month of age reach a post absorptive state in 18 hours. Mature birds according to Mitchell, must be fasted at least for 48 hours to attain a fasting level. Henry, Magee and Reid (1934) found that it takes 16 to 26 hours for a fowl to empty its alimentary canal. This would then be more or less the period necessary to fast a fowl for a basal metabolism determination, since the food stimulus will be removed. The observation of the latter workers agrees with ours in that our chickens were reduced to a fasting level after 18 hours.

GRAPH I.



In Graph I the variation of basal metabolism with age for humans, pigs and chickens has been plotted. The curve for humans has been duplicated from Du Bois (1916), while that of the pig is a reproduction of the data of Wood (1926). For comparison, a curve representing the trend of the basal metabolism in poultry has been inserted. These figures were obtained from the data of Mitchell (1927) as well as ours. Naturally the basal metabolism per square meter of one-month-old chickens reported by Mitchell (1927) is higher than ours, since the prediction formula differs. The point on the graph represented at one month has been obtained by employing the formula of Mitchell (1929) $S = 8 \cdot 19\ W^{\cdot 705}$ and utilizing our heat production values, since the values obtained in this study appear to be more representative of the basal metabolism as indicated by the R.Q.'s.

The interesting features of the curves are, that the chicken reaches its highest metabolic rate at approximately one month of age, the pig attains the same level at approximately four months, and the human between four and six years. With the chickens the basal heat production increases from hatching to one month at a rapid rate, being about 22 per cent higher at the latter age, than at hatching. From one month, the basal heat production seems to fall as rapidly as it increased and reaches a level of heat production almost equivalent to that of mature birds in two to three months time. The highest basal metabolism reached by pigs is approximately 70 per cent. more than the adult level, while with chickens it is only approximately 12 per cent. more. This difference may bear some relation to the life span of the animal and the rate at which it attains maturity.

From these results it will appear, that from three weeks to five weeks, the basal needs of chickens are at their highest, and would therefore in practice constitute the period during which they should be fed more liberally to attain good growth.

SUMMARY AND CONCLUSIONS.

1. By the application of the mold method the individual surface area of 17 chickens of approximately one month of age were determined and formulae evolved for the prediction of the surface area. The value for K in the Meeh formula was found to be 11·2, while by the method of least squares a formula equivalent to $S = 36{\cdot}31\ W^{\cdot 457}$ was obtained.

2. The basal heat production of one-month-old chickens, when expressed per square meter of body surface, was found to be 891 calories per day. If the formula of Mitchell for estimating the surface area is applied, the basal heat production becomes 1,085 calories per day.

3. It appears that the highest basal metabolism in chickens is reached between 3½ to 4½ weeks.

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Plant Proteins III.

The Supplementary Effect amongst Certain Plant Proteins.

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It is a generally recognized fact in protein studies, that the nutritive value of a certain protein feed may be significantly enhanced by the inclusion of some other protein feed in the ration. Such a supplementation amongst different proteins naturally becomes of considerable practical importance in assessing the biological values of the proteins most commonly employed in feed mixtures, since it has been shown, that the supplementary action of proteins cannot be considered to occur in a linear fashion. Consequently it cannot be assumed that the mean of the biological values of two proteins at a certain level of feeding, would represent the biological value of a mixture of equal parts of the respective proteins. Conclusive proof as to whether supplementation between two proteins has actually taken place, can only be obtained by a direct determination of the biological value of each protein, as well as the mixture of the two proteins at the same level of intake.

Supplementation amongst proteins is the nett result of a variable amino acid constitution of protein feeds in respect to the indispensable amino acids. A protein fed at a low level of intake may be considered to consist of two distinct entities, one constituting the amino acids necessary for maintaining the integrity of the tissues and the other composed of those amino acids which form an incomplete assortment, and consequently incapable of promoting synthesis of new tissues. These latter amino acids are therefore drawn into the oxidative processes of the body and catabolized. If, however, a second protein, which has a different indispensable amino acid deficiency, is fed in conjunction with the first, it can readily be seen how the fractions of each protein which separately were destined to be deaminized and oxidized can between them make up a complete assortment of those indispensable amino acids to satisfy the requirements of new tissue and hence promote synthesis. In this case the biological value of the mixture of the two proteins will obviously be greater than the mean biological value of the two proteins, since a portion of the two fractions in the respective proteins, which would otherwise have gone waste as far as tissue synthesis is concerned, is now utilized in the body.

It is evident from the above reasoning, that supplementation will not take place between all proteins. If the remaining fraction of each protein in the mixture, after maintenance has been taken care of, should be deficient in the same indispensable amino acid, then naturally there exists no chance of selecting a complete amino acid complex for synthetic purposes and consequently no supplementation is possible. On the other hand it is self evident that the greater the variation of the missing indispensable amino acids in the two fractions, which effect supplementation, the greater are the possibilities of a supplementation. A protein having a biological value of 80 would, therefore, indicate that 20 per cent. of the absorbed nitrogen has not been utilized on account of a deficiency of one or more of the indispensable amino acids in the protein molecule. Another protein having a biological value of 50, shows that 50 per cent. of the absorbed nitrogen has not been used by the body. In the latter case the fraction which can be supplemented is much greater than the first. If, therefore, these two proteins are fed as a mixture and supplementation takes place, then one has to assume that it was possible for the body to select a complete assortment of amino acids from the 20 and 50 per cent. fractions.

In a previous paper [Smuts and Malan (1938)] the biological values of individual plant proteins have been determined. In this study the supplementary effect amongst certain of these proteins is reported on.

As far as the authors are aware no studies have been conducted on the supplementary effect of the proteins investigated in this study. However, since the supplementation can only be accomplished when the limiting indispensable amino acids of a protein are covered by the inclusion of a second protein, it seems appropriate at this stage to refer to some investigations concerned with the amino acid deficiencies of the proteins under discussion. Haag (1931) in a study on the physiological effects of rations restricted principally or solely to plants, claims that lucerne is seriously deficient in cystine for growth in rats. Scoz (1932) found that cystine deficiency limits growth in rats, and that the animal protects the protein sulphur of the organism at the expense of less essential substances. It would appear, therefore, that lucerne is deficient in the indispensable amino acid cystine and that it can consequently only be supplemented by the addition of cystine or another protein containing enough cystine or methionine to cover the cystine deficiency [Jackson and Bloch (1933); Weichselbaum (1935)].

Morris and Wright (1933) in a study on the protein values for milk production claim that peanutmeal is deficient in lysine and since this amino acid is considered ~~necessary~~ for milk production, peanutmeal ranks low as a protein feed for lactating animals. On the other hand John and Jones (1917) by chemical analysis showed that the protein of peanut is rich in lysine. Quite recently Beach and White (1931) stated that the protein of arachin is deficient in methionine and that L.-cystine was incapable of replacing the former.

McCollum and Simmonds (1916) as well as Mitchell and Smuts (1932) have shown that oats are deficient in the indispensable amino acid lysine. Outhouse and Krause (1934) found that a lysine deficiency with rats causes an inefficient utilization of protein nitrogen and a less active cellular metabolism.

THE PAIRED FEEDING TESTS.

Paired feeding tests together with the determination of the respective biological values were conducted on the different rations. The rations were compounded in such a fashion that they were, as far as possible, equal in all respects except for the difference in source of protein. In the comparison of peanut plus lucernemeal with lucernemeal plus cystine, 7 per cent. of agar replaced an equal weight of starch in the former ration, while in the comparison of peanut and peanut plus oats no agar was included in the ration. The different proteins were incorporated in the ration so as to furnish approximately 9 per cent. of crude protein. The other constituents included in the ration contributed negligible amounts of nitrogen but supplied the necessary amounts of other nutrients to make the rations complete. The comparison of the rations is given in Table 1.

TABLE I.

Composition of Rations.

	A.	B.	C.	D.	E.	F.
Peanutmeal	16·5	—	—	7·8	7·8	—
Lucernemeal	—	57·1	55·7	—	28·6	—
Oatmeal	—	—	—	36·4	—	—
Cystine	—	—	·20	—	—	—
Ext. Egg white	—	—	—	—	—	3·8
Sucrose	10·0	10·0	10·0	10·0	10·0	10·0
Butterfat	8·0	8·0	8·0	8·0	8·0	8·0
Yeast Extr. 1	10·0	10·0	10·0	10·0	10·0	10·0
Agar 3	—	—	—	—	—	2·0
Codliveroil	2·0	2·0	2·0	2·0	2·0	2·0
NaCl	1·0	1·0	1·0	1·0	1·0	1·0
Salt Mixture 2	4·5	4·5	4·5	4·5	4·5	4·5
Starch	48·0	7·4	8·6	20·3	28·7	58·7
TOTAL	100·0	100·0	100·0	100·0	100·0	100·0
Per cent. Nitrogen	1·45	1·52	1·55	1·42	1·50	·650

(1) Yeast extract was prepared according to the method of (Itter 3, Orent E. R. and McCollum E. V. J. B. C. 108, 2, 571–577, 1935.)

(2) A modified Osborne and Mendel salt mixture, proposed by P. B. Hawk and B. L. Osler, 1931, Science Vol. 74 p. 369.

(3) The fibre content was equalized by adjusting the agar in the rations compared.

Five to six pairs of rats were used in the different paired feeding tests. These were selected according to age, sex, litter and weight. Each pair received the same amount of food. The initial weights taken over three consecutive days, together with the total gains, total food consumption, and total gain per week of the different comparisons, are summarized in tabular form.

In Table II a summary of the comparisons between lucerne supplemented by ·20 per cent. cystine and lucerne meal is given. In each pair the rat receiving the cystine supplement exceeded its mate in total gain in weight over the experimental period. Such a consistent outcome favouring either one or the other ration would have resulted from chance only twice in 128 trials. The average difference in gain between the six pairs is 13·5 with a standard deviation* of 6·5. According to Students probability table, when Z which is equal to $\frac{\text{M}}{\text{St. Deviation}}$ is equal to 2·0 and $N=6$, the chances are approximately one in 300 that this difference is due to chance. From a comparison of the weekly gains, it is seen that out of the 42 weekly comparisons, the cystine supplemented rat exceeded its pairmate in gains in 29 cases. This experiment, therefore, clearly demonstrates a difference between the two rations, and since the only difference was the inclusion of cystine in one ration, which proved to be superior to the control ration of lucernemeal, it must be concluded that the addition of cystine was instrumental in promoting more rapid growth.

From Table III it is evident that when peanut is supplemented by oatmeal, the resulting protein mixture is superior to that of peanut alone. Out of the 30 weekly comparisons of gains, 18 favoured the peanut plus oatmeal, while only 9 favoured the peanut ration. The average difference in total gains is 9·8 and the standard deviation 4·7. The ratio (Z) of the mean difference to the standard deviation is 2·1. With $Z=2{\cdot}1$ and $N=5$, the odds according to Students' table, that the addition of oatmeal to peanutmeal was responsible for the more rapid gains experienced on the mixed protein ration, are approximately 143 to 1.

NITROGEN METABOLISM EXPERIMENTS.

The same rations as were used in the paired feeding tests were utilized in the nitrogen metabolism experiments. An extra period consisting of lucernemeal supplemented by peanut in equal proportions in respect to protein was also included. The nitrogen low ration was adjusted for its fibre content by the addition of agar to equal that of the protein ration under investigation. The collection periods were of 7 days duration and were in each case preceded by a preliminary feeding period of 6 days on the same ration. For the biological value determination of each protein a new series of rats were used, so that rats were not kept longer than 26 days on experiment.

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* Standard Deviation = $\sqrt{\frac{\Sigma D^2}{n-1}}$

Summary of Bodyweights, Gains, Feed Records, Number of Refusals, and Total Gains per week on Lucerne and Lucerne plus Cystine Ration.

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.	Lucerne and Cystine.	Lucerne meal.
Initial wt............	64·0	66·0	70·0	63·0	92·0	92·0	53·0	52·0	54·0	49·0	60·0	54·0
Final wt............	140·0	120·0	142·0	117·0	135·0	130·0	122·0	104·0	105·0	89·0	108·0	94·0
Gain in grams........	76·0	54·0	72·0	54·0	43·0	38·0	69·0	52·0	51·0	40·0	48·0	40·0
Total Food consumption	509	509	530	530	474	474	493	494	407	407	410	410
Refusals............	0	6	0	4	4	1	1	8	0	16	0	15
Total gains per week..	7—0—0		6—1—0		3—2—2		4—2—1		5—1—1		4—1—2	

TABLE III.

Summary of Bodyweights, Gains, Feed Records, Refusals and Gains per week on Peanut and Peanut plus Oatmeal Rations.

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		
	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	Peanut meal.	Peanut and Oatmeal.	
Initial wt............	9·50	97·0	92·0	99·0	94·0	87·0	97·0	99·0	97·0	105·0	
Final wt............	130·0	142·0	125·0	140·0	124·0	132·0	124·0	139·0	126·0	137·0	
Gain...............	35·0	45·0	33·0	41·0	30·0	45·0	27·0	40·0	29·0	32·0	
Total Food consumption	356	356	369	369	334	334	339	339	322	322	
Refusals............	5	0	1	1	4	0	4	2	3	2	
Total gains per week..	3—0—3		3—0—3		2—0—4		0—2—4		1—1—4		9—3—18

TABLE IV.

Nitrogen Metabolism Data and the Calculation of Biological Values.

Animal No.	Initial weight grm.	Final weight grm.	Average weight grm.	Daily food intake grm.	Daily N intake mgm.	Daily Fecal N mgm.	Body N in Feces.		Food N in Feces mgm.	Absorbed N mgm.	Daily Urinary N mgm.	Body N in Urine.		Food N in Urine mgm.	Food N Retained. mgm.	Biological Values.
							Per grm. food consumed. mgm.	Per day mgm.				Per 100 gm. weight mgm.	Per day mgm.			
N. Low Period for Lucerne plus Peanut Ration.																
30	91·0	92·0	91·5	8·0	—	25·55	3·20	—	—	—	22·94	25·07	—	—	—	—
31								—	—	—			—	—	—	—
32	92·0	92·0	92·0	9·3	—	26·97	2·90	—	—	—	22·48	24·44	—	—	—	—
33	104·0	104·0	104·0	8·0	—	25·55	3·22	—	—	—	24·98	24·02	—	—	—	—
34	83·0	84·0	83·5	7·6	—	25·17	3·31	—	—	—	20·44	24·48	—	—	—	—
35	91·0	91·0	91·0	6·6	—	16·59	2·51	—	—	—	23·74	26·09	—	—	—	—
Lucerne plus Peanut Ration containing 1·50 per cent. Nitrogen.																
30	89·0	97·0	93·0	10·6	159·0	53·80	3·20	33·92	19·88	139·12	64·18	25·07	23·32	40·86	98·26	71
32	99·0	115·0	107·5	10·9	163·5	50·06	2·90	31·61	18·45	145·05	76·64	24·44	26·37	50·27	94·78	65
33	10·0	109·0	105·0	10·4	156·0	55·06	3·22	33·49	21·57	134·43	69·94	24·02	25·22	44·72	89·71	67
34	90·0	95·0	92·5	10·9	163·5	50·50	3·31	36·08	14·42	149·08	74·24	24·48	22·64	51·60	97·48	65
35	95·0	98·0	96·5	10·9	163·5	65·97	2·51	27·36	38·61	124·89	64·24	26·09	25·18	39·06	85·83	69
															Average......	67
N. Low Period for Oatmeal plus Peanut Ration.																
36	120·0	115·0	117·0	6·2	—	24·41	3·94	—	—	—	25·72	21·98	—	—	—	—
37	110·0	112·0	111·0	7·6	—	26·14	3·44	—	—	—	17·16	15·45	—	—	—	—
38	111·0	112·0	111·5	6·7	—	18·29	2·73	—	—	—	23·98	21·50	—	—	—	—
39	110·0	110·0	110·5	8·0	—	25·76	3·22	—	—	—	21·78	19·71	—	—	—	—
40	110·0	105·0	107·0	5·4	—	18·04	3·33	—	—	—	26·70	24·95	—	—	—	—
41	105·0	102·0	103·5	5·7	—	20·24	3·55	—	—	—	23·32	22·53	—	—	—	—

TABLE IV (*Continued*).

Oatmeal plus Peanut Ration containing 1·42 per cent. Nitrogen.

Animal No.	Initial weight grm.	Final weight grm.	Average weight grm.	Daily food intake grm.	Daily N intake mgm.	Daily Fecal N mgm.	*Body N in Feces.*		Food N in Feces mgm.	Absorbed N mgm.	Daily Urinary N mgm.	*Body N in Urine.*		Food N in Urine mgm.	Food N Retained. mgm.	Biological Values.
							Per grm. food consumed. mgm.	Per day mgm.				Per 100 gm. weight mgm.	Per day mgm.			
36	118·0	125·0	121·5	7·7	109·3	25·67	3·94	30·34	0·0	109·3	48·8	21·98	26·71	22·09	87·21	80
37	116·0	118·0	117·0	7·1	100·8	24·38	3·44	24·42	0·0	100·8	39·2	15·45	18·08	21·12	79·68	79
38	118·0	127·0	122·5	8·0	113·6	21·81	2·73	21·85	0·0	113·6	50·9	21·50	26·34	24·56	89·04	78
39	122·0	125·0	123·5	6·7	95·1	23·10	3·22	21·57	1·53	93·6	39·8	19·71	24·34	15·46	78·14	83
40	119·0	123·0	121·0	8·0	113·6	25·15	3·33	26·64	0·0	113·6	46·6	24·95	30·19	16·41	97·19	85
41	112·0	122·0	117·0	8·0	113·6	23·61	3·55	28·40	0·0	113·6	47·2	22·53	26·36	20·84	92·76	82
															Average.........	81

N- Low Period for Lucerne plus Cystine Ration.

Animal No.	Initial weight grm.	Final weight grm.	Average weight grm.	Daily food intake grm.	Daily N intake mgm.	Daily Fecal N mgm.	Body N in Feces per grm. food consumed mgm.	Body N in Feces per day mgm.	Food N in Feces mgm.	Absorbed N mgm.	Daily Urinary N mgm.	Body N in Urine per 100 gm. weight mgm.	Body N in Urine per day mgm.	Food N in Urine mgm.	Food N Retained. mgm.	Biological Values.
50	127·0	122·0	124·0	6·4	—	26·94	4·21	—	—	—	21·87	17·63	—	—	—	—
51	130·0	132·0	131·0	5·7	—	25·14	4·41	—	—	—	22·48	17·16	—	—	—	—
52	114·0	114·0	114·0	7·8	—	42·20	5·41	—	—	—	22·48	19·71	—	—	—	—
53	110·0	110·0	110·0	6·8	—	35·43	5·21	—	—	—	18·48	16·81	—	—	—	—
54	100·0	100·0	100·0	7·7	—	35·57	4·62	—	—	—	19·09	19·09	—	—	—	—
55	105·0	105·0	105·0	6·8	—	29·87	4·39	—	—	—	19·40	18·47	—	—	—	—

Lucerne plus Cystine Ration containing 1·55 per cent. Nitrogen.

Animal No.	Initial weight grm.	Final weight grm.	Average weight grm.	Daily food intake grm.	Daily N intake mgm.	Daily Fecal N mgm.	Body N in Feces per grm. food consumed mgm.	Body N in Feces per day mgm.	Food N in Feces mgm.	Absorbed N mgm.	Daily Urinary N mgm.	Body N in Urine per 100 gm. weight mgm.	Body N in Urine per day mgm.	Food N in Urine mgm.	Food N Retained. mgm.	Biological Values.
50	115·0	129·0	122·0	9·0	139·5	66·0	4·21	37·89	28·11	111·39	35·75	17·57	21·44	14·31	97·08	87
51	127·0	140·0	133·5	9·0	139·5	68·0	4·41	39·69	28·31	111·19	32·34	17·16	22·91	9·43	101·76	91
52	108·0	124·0	116·0	9·0	139·5	68·2	5·41	48·69	19·51	119·99	31·27	19·71	22·86	8·41	111·58	93
53	104·0	120·0	112·0	9·0	139·5	65·1	5·21	46·89	18·21	121·29	31·60	16·81	18·83	12·77	108·52	89
54	100·0	90·0	95·0	8·0	124·0	63·4	4·62	36·96	26·44	100·91	36·08	19·14	18·18	17·90	83·01	82
55	72·0	110·0	91·0	9·0	139·5	62·6	4·39	39·51	23·09	116·41	37·82	18·47	16·81	21·01	95·40	82
															Average.......	87

As will be seen from Table IV the individual biological values of the lucerne peanut mixture varied from 63 to 71 with an average value of 67. Since the biological values of lucerne and peanutmeal as determined in a previous paper [Smuts and Malan (1937)] are 61 and 69 respectively, the value of 67 obtained for the mixture of these two proteins, at approximately the same level, can only be interpreted as indicating a non-supplementary effect.

In the case of lucernemeal supplemented by ·20 per cent. cystine, the individual biological values vary from 82 to 93 with an average figure of 87. This would mean that the addition of ·20 per cent. cystine to lucerne increased the utilization of its absorbed nitrogen by 27 per cent. When peanutmeal is supplemented by oatmeal, in equal proportions in respect to protein, there seems to be a distinct supplementation between these two proteins. The biological value of peanut and of oatmeal as determined in a previous paper (Smuts and Malan 1937) was found to be 69 and 84 respectively. If no supplementation took place one would expect the biological value of the mixture to be 77. This value is lower than the determined biological value of the mixture, namely 81. It seems therefore reasonable to deduce that supplementation has taken place. This conclusion is, furthermore, supported by the outcome of the growth studies in which the protein mixture of peanut plus oats is significantly superior to peanut alone, when fed at the same level of protein intake. Mitchell and Smuts (1932) have shown that oats are deficient in lysine. Since supplementation has taken place between oatmeal and peanutmeal, it seems obvious that peanutmeal must have been able to cover the lysine deficiency in oats. In a later paper it will be shown by means of paired feeding tests, that the addition of lysine did not enhance the growth promoting properties of peanutmeal.

SUMMARY AND CONCLUSIONS.

By means of the paired feeding method, it has been shown that the addition of cystine to lucerne significantly enhances the growth promoting properties of the latter, and that when peanutmeal is supplemented by oatmeal the resulting protein mixture is superior to that of peanutmeal alone.

Nitrogen metabolism studies conducted on the same rations showed that the incorporation of ·20 per cent. cystine in a lucerne ration definitely increased the biological value of lucerne, that no supplementation exists between peanutmeal and lucernemeal, and that supplementation occurs between peanutmeal and oatmeal.

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The Influence of Frequency of Cutting on the Yield, Chemical Composition, Digestibility and Nutritive Value of some Grass Species.*

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I. INTRODUCTION.

The steadily increasing volume of literature on pasture research bears mute testimony to the interest taken by agricultural workers all over the civilised world in problems relating to the pastoral resources of their respective countries. This is not surprising in view of the fact that pasture represents the raw material for the manufacture of the most important animal products, such as milk, meat, wool, and hides.

Problems associated with pasture research become imperative in a country such as the Union of South Africa where " more than 85 per cent of the land is unfit for cultivation, being too dry, mountainous, or otherwise unsuitable, and can be utilised only for pasturage " (Leppan, 1928, 1936). In this connection it may be pointed out that the rainfall of South Africa varies from about 50 inches on the Natal coast to less than 2 inches at Luderitzbucht on the west coast, whereas fully half of the Union receives less than 20 inches per annum, and only about a quarter receives more than 25 inches. The problems confronting the pasture investigator are consequently many and varied.

An important preliminary to intensive pasture investigations is exact knowledge with regard to the plant population of a country. The inception of a Botanical Survey of the Union in 1918, under the supervision of the Chief of the Division of Plant Industry was, therefore, a step in the right direction. As a result of this work, in conjunction with that of earlier botanists, it became possible soon afterwards to divide the country into some nineteen main botanical regions (Pole-Evans, 1920). This work is still proceeding and aims at recording and mapping the chief types of plants in each of the different botanical regions. At the same time the necessity was felt for establishing permanent stations for veld research in these regions and several such stations have been founded, but it is not intended to follow their development in detail. Suffice it to state that these experiment stations are studying problems of pasture research directly associated with the areas in which they are situated.

A great stimulus was furthermore given to pasture research by the decision of the Union Government in 1933 to combat soil erosion along national lines, for it is well known and appreciated that soil erosion and veld deterioration are closely related. A national scheme for pasture improvement and management thus came into being. The Devision of Plant Industry is mainly responsible for this work, and a review of their activities has been published by Pole-Evans (1936).

Problems pertaining to the pastoral resources of a country can best be solved with the full co-operation of the botanist, the chemist, and the animal nutritionist. Whilst, however, the botanist will be primarily interested in " the botanical composition of the vegetative covering, the habitat-range and limiting factors of the major pasture species, the factors which govern the destiny of the grassland associations, and the procedure necessary to avoid undesirable successions ", the biochemist, on the other hand, will be interested primarily in the chemical composition of the pasture and the effects thereupon of external factors such as rainfall and stage of growth. In conjunction with the nutritionist the biochemist is interested in the effects of the pasture upon the animal. He studies the chemical composition of the herbage to determine to what extent this can be correlated with its feeding value. To what extent, for instance, is the low value of certain types of grazing due to deficiency of one or more of the nutrients now known to be essential constituents of the diet. Developments in the science of nutrition have resulted in the recognition of the fact that animals are more likely to suffer from a lack of some inorganic salts, proteins under certain conditions, or of certain vitamins which are required in comparatively small amounts, than from an insufficiency of the energy-yielding and fat-forming carbohydrates.

The value of chemical composition as a means of assessing the feeding value of pastures has been realised for a long time. As early as 1873 Müller suggested the chemical analysis of German pastures as a means of determining their feeding value. In their investigations into the cause and prevention of Lamziekte in Bechuanaland Theiler and his associates (1920) were able to demonstrate the deficiency of phosphate in some South African pastures and the necessity for supplementing the natural grazing with phosphate in one form or another. As a direct outcome of this classical work Henrici (1928, 1930) undertook a more detailed study of the phosphorus content of grasses in the Vryburg district and parts of the Eastern Transvaal. Somewhat later, in 1930, the Director of Veterinary Services inaugurated a series of monthly surveys of the pastures of the Union of South Africa with the object of ascertaining the extent of phosphorus deficiency in the pastures of the country as a whole. At the same time these surveys aimed at finding out whether deficiencies other than phosphorus also occur in the natural pastures, and at collecting data on the feeding value of natural pastures growing in different areas under different environmental conditions. In this way a mass of data on the chemical composition of samples of natural pasturage has accumulated at the Veterinary Research Laboratory at Onderstepoort. Some of this information has been published by du Toit and his associates (1932, 1935), and a paper on the results of analysis of a further four thousand samples is being prepared for publication.

These samples were obtained in the field in different areas by following the grazing animals and collecting the samples as selectively as the grazing animal followed selected its food. It soon became apparent, however, that pasture samples at all stages of growth were being received during any one month of collection with the doubtful exception of the winter collections from the greater part of the area covered when mainly mature, dry grass was received. The analyses therefore varied as the stages of growth did for different samples and it was soon realised that more definite knowledge on the influence of stage of growth on the chemical composition of grasses was needed.

With this object in view species of indigenous grasses were established in pure stands on small plots and subjected to various systems of defoliation ranging from monthly cuts to one cut in every twelve months. Preliminary results of these investigations were published by du Toit et al (1934, 1935), and indicated inter alia that grasses which were allowed to grow undisturbed through the whole of the growing season and then left to dessicate on the veld became so low in their phosphorus and protein contents that animals which have to subsist entirely on such material would not ingest sufficient of these nutrients for maintenance requirements. Even aftermath growth, i.e. growth following upon a defoliation in February or March was relatively poor in phosphorus and protein if allowed to become dry on the veld. These results forced the conclusion that more and better use must be made of the produce of the growing season.

Chemical composition in itself cannot, however, be of much assistance unless it can be correlated with nutritive value. Unfortunately the determination of the latter entails much more work than the former in that actual feeding experiments involving costly equipment need to be carried out with animals. As a first step digestibility data are of great importance, and, as pointed out recently by Ross (1936), " it is surprising how little has been done in this direction ". While it is realised that work done in other countries on the chemical composition of pastures in relation to their nutritive value may be of great assistance to us in estimating the value of our pastures from data on chemical composition, the differences in soil type and fertility, flora, and climatic conditions, resulting in differences in the chemical composition of the vegetation, necessitate undertaking of similar work in the Union.

The chief shortcomings of South African pastures are to be found mainly in the long periods of dormancy. In the summer-rainfall areas of the Union the dry, cold winter months cause almost complete cessation of growth. The growing season commences with the first spring rains, usually in September, and continues with varying intensity to about the end of April. The continued provision of nutritious grazing during this period could be accomplished by introducing a system of rotational grazing. The adoption of a system which would produce optimum results presupposes, however, an understanding of the factors influencing yield, chemical composition, and nutritive value of the pasture.

On the other hand, if the deterioration of the herbage in regard to its nutritive qualities during the dormant season is borne in mind, and if it is admitted that farming in many parts of the country can be stabilised only when droughts are considered normal occurrences, supplementary feed in one form or another will have to be provided; during the dry season if continued production is aimed at, and during times of drought if heavy losses of stock are to be prevented.

The provision of supplementary feed could be accomplished in several ways. The utilisation of part of the annual maize crop for this purpose has been repeatedly advocated. The feasibility of combining rotational grazing with cutting for hay, thereby conserving any excess growth in a nutritious condition for the time of scarcity could with advantage be explored. In this connection reference may be made to a series of experiments conducted at the Grootfontein School of Agriculture, Middelburg (Maré, 1937), and which aimed at ascertaining data on the influence of different systems of grazing by sheep on the permanency and productivity of an established lucerne pasture. By combining a system of rotational grazing with cutting for hay a uniform carrying capacity of 24 sheep per morgen during summer and winter was obtained for a period of three successive years.

The problem of improving and supplementing the poor and inadequate grazing of summer and autumn in the areas of winter-rainfall and summer drought is receiving much attention at the Stellenbosch-Elsenburg College of Agriculture (Marais and Sim, 1937). Lucerne, which was formerly considered suitable only for cultivation on irrigable soil, has been successfully established under dryland conditions at Elsenburg. Such a lucerne pasture was found to provide excellent grazing during May, June and July, the time of the year when other forage crops and the natural veld are as yet too immature to be grazed or cut for silage. Furthermore the first spring growth may be cut and conserved as silage or hay, after which the lucerne may be grazed until January. The possibilities of lucerne in the drier parts of the winter rainfall area are also being investigated at that Institute.

In many of the better favoured areas of the Union the production of forage crops under irrigation will certainly be of importance in providing supplementary feed, but unfortunately the possibilities for irrigation schemes are limited. On the other hand, certain regions of the summer rainfall area are blessed with a rich grass flora which grows rapidly during the rainy season and, if not grazed down regularly, soon reaches a stage of maturity where it is unpalatable and of low nutritive value. By applying scientific methods of management some of this growth can be made to contribute towards the provision of supplementary feed during the times of scarcity. A possible system is considered to be the reservation of certain level areas on the farm solely for the purpose of cutting for hay at suitable intervals during the growing season.

The investigation to be reported on in the following pages is an attempt to obtain information on certain phases of the possibility mentioned above and aims at finding an answer to the question: How often should grasses be cut for hay during the growing season with the highest possible benefit to animals which have to be fed for maintenance or production of the material so obtained, and, at the same time, with the least possible injury to the vitality of the plants which have to continue yielding nutritious herbage during succeeding growing seasons.

II. REVIEW OF THE LITERATURE.

A review of the literature bearing upon the subject of pasture research would form an important investigation in itself, and would be out of place in an article of this nature. Only a limited number of references have been collected from available sources, and it is hoped that these will prove adequate for the purpose of illustrating some of the earlier work and the present state of our knowledge.

The idea that the nutritive value of pasture is dependent upon its chemical composition is not new. The suggestion of Müller (1873) that the chemical analysis of German pastures should be undertaken as a means of determining their feeding value has previously been referred to. Towards the end of the last century the available data on the chemical constituents of plant ash were compiled by Wolff (1880). In more recent times Prof. E. Mangold of Berlin inaugurated a series of investigations into the mineral composition of the most commonly used German fodders. The inaugural dissertation of Theel (1933) on the mineral content of meadow hays may be quoted as an example of this work. This investigator was unable to find a correlation between the mineral and organic constituents of hay, a protein-rich hay being often deficient in mineral matter.

According to Orr (1929) the first systematic work on the chemical composition of plants was undertaken by Lawes and Gilbert (1856-1900). Detailed analyses of the ash were made and the results showed that the chemical composition of the mixed herbage was closely dependent upon its botanical composition and the stage of development of the plants. Orr states that "it is interesting to note that work on these grass plots laid out at Rothamsted in 1856 is still being continued". An account of the more recent work at that station is given in a monograph by Brenchley (1924).

Some of the earliest investigations into the chemical composition of South African feeding stuffs were made by Juritz (1890) when he published the results of a few analyses of some of the principal fodder plants of the Karroo. Similar work was continued by him (Juritz 1914) and others [Vipond (1914) and Marchand and Smit (1919)]. However, no systematic investigation of South African pastures was undertaken until after the results of the work of Theiler and associates (1920) on Lamziekte in relation to phosphorus deficiency became known. In fact, it may be stated in general that interest in the mineral composition of pastures has been largely influenced by development in our knowledge of the food requirements of animals. Since certain pastures had been proved to be so poor in one or other constituent that this deficiency may cause disease in the grazing animal, it became a matter of economic necessity to study the composition of natural pastures, ~~and~~ the factors having an influence on that composition. The problem was considered to be of such importance that as a result of the answers received from the various Governments within the British Commonwealth of Nations to a questionnaire issued by a sub-committee of the Civil Research Committee of the British Cabinet, the whole question of the mineral content of the pastures was reviewed by Orr (1929) in a monograph containing all the available information bearing on the subject in an easily accessible form.

At the suggestion of Major Elliot (1926) a systematic investigation of the chemical composition of pastures was begun at the Rowett Institute, Aberdeen, in 1923. Samples of pasture were collected from many grazing areas in Britain and from some overseas parts of the Empire. From data so accumulated Godden (1926) was able to show wide differences in the mineral content of pastures of different origin. The following table given by Godden may be quoted as an illustration of the dry matter composition of poor and rich pastures:—

	% CaO	% P_2O_5	% Na_2O	% K_2O	% Cl	% N
Island of Lewis—						
Eaten	·286	·243	·377	·678	·115	1·340
Not eaten	·295	·177	·379	·540	·102	1·029
Falkland Islands	·225	·488	·290	1·980	·580	1·650
Taplow Pastures highly treated	2·473	·997	·698	2·398	·498	3·562
Average for cultivated pastures	1·004	·735	·246	3·177	·950	2·830

The herbage of untreated natural pastures was found to be markedly poorer than that of cultivated pastures in respect of minerals and nitrogen, while the percentage of mineral constituents in the ash of " not eaten " grass from the hill pastures were only about 50 per cent. of those in the ash of " eaten " grass. Sheep thus appeared to eat, by preference, the herbage which contained the higher percentage of mineral ingredients. It was further shown that in spite of marked differences in the mineral content of the different types of pasture there was little difference in their caloric value as calculated.

In South Africa Taylor (1922) collected samples of grass species occurring most commonly on the veld of the Cedara experimental farm at approximately the same period of each year. These samples were taken when the grasses were in the early flowering stage, and the phosphate (P_2O_5) content of the average veld grass was found to be very constant at about 0·2 per cent. of the dry matter. Henrici (1928) observed that the phosphate content of grasses fluctuated to a marked degree in the course of the year, and that the Armoedsvlakte grasses had a phosphate content only one-half to one-sixth of that of European grasses. A similar state of affairs existed at Ermelo (Henrici, 1930), although the rainfall in this district was higher than at Armoedsvlakte. The same investigator (1932, 1934, 1935) continued her work on the mineral composition of natural pastures in various other parts of the country. Everywhere she found grasses to be poor in phosphate content when compared with values for European cultivated pastures. Sodium and nitrogen were also found to be distinctly low in some cases. On the other hand, the bushes of the Eastern Cape Province were higher in protein content than the grasses and most of them were rich in phosphorus and calcium.

Du Toit and co-workers (1932, 1935) analysed many thousands of samples of natural pasturage from different areas in the Union in addition to studying the mineral and protein content of individual grass species at different stages of growth. They concluded that practically all South-African pastures were low in phosphorus for the greater part of the year. Different species of grasses grown on the same soil showed remarkable variations in composition at the same stage of growth, while the percentage of certain constituents, phosphorus, potassium, nitrogen, and chlorine, decreased to a remarkable extent in all species as the period of growth advanced beyond one month. Crude protein varied more or less directly with the phosphorus content and is extraordinarily low during certain seasons of the year. Generally the sodium content was so low that it suggested an insufficiency of this constituent for producing animals on pasture.

A considerable amount of attention has been devoted to the factors affecting the yield, mineral, and protein content of pasture species as such studies were regarded as fundamental to any extensive work in the field. In this connection the work done by Australian investigators deserves to be mentioned. Richardson, et al (1931) found that pure species grown under similar soil conditions in controlled pot cultures and harvested at a definite stage of growth showed wide differences in mineral and nitrogen content and in relative yielding capacity. Growth stage was found to exercise a determining influence on the nitrogen and mineral content of pasture species. Nitrogen assimilation and the absorption of essential nutrients were most active at the tillering stage, while photosynthesis rose to a maximum shortly after the flowering stage. The percentage of nitrogen, phosphoric acid, potash, and to a lesser extent, calcium and magnesium in these plants thus fell rapidly from the early tillering stage to maturity. The application of soluble phosphate did not materially affect the phosphorus content of plants grown on fertile soil. On the other hand, dressings of superphosphate on phosphorus deficient soil increased the phosphorus content and the yield of the herbage. Davies and Sim (1931) obtained the highest yield of dry matter from a natural pasture by allowing the herbage to reach maturity, more frequent cutting tending to reduce the yield. However, three cuts during the growing season yielded approximately 94 per cent. of the dry matter obtained from one cut, and produced herbage of higher nutritive value than that of mature herbage.

Through these investigations it has been shown that while the environment expressed in terms of climate, soil composition, and pasture management exerted an important influence on the mineral content of the individual plant, there was a limit to the ability of plants to make use of available plant nutrients, which was imposed by the nature of the species itself. The necessity for further work on pure species in regard to their suitability for specific environmental conditions with a view to introducing such species into the permanent pastures was obvious. Applying varying systems of defoliation to a perennial grass, *Phalaris tuberosa* which was found to be persistent and of high yielding capacity in the Australian zone of winter rainfall and summer drought (Trumble and Davies, 1931) Richardson, et al (1932) concluded that a system of three cuts during the growing season would probably give optimum results, if the yield of dry matter, the yield of nutrients, and the permanence of the plant in an arid environment were taken into consideration.

Cashmore (1934) made a comparative study of *Lolium perenne* and *Phalaris tuberosa*, and found that the latter was the more persistent and more productive species when once established. Preliminary work involving trials of species, strains, and mixtures of herbage plants suitable for an irrigated reclaimed swamp has been started by Trumble and Davies (1934). They indicated that the yield of mixtures containing perennial rye-grass, cocksfoot, white and red clover was approximately double that of the best perennial rye-grass pure swards.

A clear picture of the various projects in grassland research being studied in Australia at the present time is obtained by a perusal of a bulletin recently published by the Imperial Bureau of Plant Genetics, Aberystwyth, Wales (1934).

According to McConkey (1931) grassland products form 94 per cent. of the total exports of New Zealand. In view of this fact and considering that the country is blessed with a well distributed annual rainfall it is not surprising to learn that " nowhere in the world is the standard of pasture management higher than in New Zealand ". (Grassland Research Committee, Pretoria University—African Explosives and Ind. Ltd. 1932). However, while the favourable climatic conditions were no doubt a contributory factor, the investigations by Cockayne, Bruce Levy, Hudson, and others (1933) on the testing of species and strains which would prove most suitable for the environment, and the influence of fertiliser and management on the productivity of the pastures, have been and will be the main factor in deciding on the most efficient methods of utilising the herbage.

Through their researches into the chemical composition and yield of individual species of grasses Fagan and Jones (1923) did much to clarify thought on some aspects of pasture management. Attention was focussed on the differences in yielding capacity and chemical composition of individual species. It was shown that the total protein produced by hay and aftermath was less than that given by a smaller yield of several pasture cuts. The contradictory results in regard to the yield of dry matter obtained by Stapledon et al (1925-26), who found that the yield of Italian Rye-grass was increased with increasing number of cuts was attributed by them partly to the fact that this species recovered very quickly after cutting or grazing.

Taylor (1929, 1932) also studied the influence of season and fertilizer on the yield and chemical composition of pasture under different intensities of grazing. His conclusions were in the main the same as those arrived at by other investigators. A few points of special interest may, however, be referred to. It was found that the protein content of *Themeda triandra var. Burchellii*, the predominant species of the Rooigras veld of Natal, was considerably lower than that of European grasses after a similar period of growth, the figures for fort-nightly and monthly cuts being on an average 12·25 per cent. and 10·0 per cent. of the dry matter, respectively. As pointed out by Paterson (1933, 1935) the more rapid growth of plants in tropical regions as opposed to growth in the temperate zones of the world may produce a much quicker drop in the percentage of protein. Further, cutting the herbage at fort-nightly intervals killed so many plants that it was not possible to continue the experiment on the same quadrats during the following season. Du Toit et al (1935) found that cutting at monthly intervals may also prove at times too drastic for the vitality of the plants.

Prior to the now well known work of Woodman and his co-workers (1926, 1927, 1928, 1929, 1931, 1932) of the Animal Nutrition Institute, Cambridge, little was known in regard to the digestibility of grass at the pasture stage of growth. This work is fundamental to a knowledge of intelligent pasture management and the feeding of stock on pasture. The yield, chemical composition and digestibility of pasture after periods of growth ranging from one week to five weeks were studied. It was found that pasture cut at weekly intervals had a protein content around 25 per cent. throughout the whole season, and a digestibility of over 80 per cent. In fact, the digestibility and nutritive value of such closely grazed pasture compared very favourably with that of a concentrate such as linseed cake, and if supplementary food was needed on pasture of this nature it should be given in the form of a carbohydrate-rich food which would balance the ration. Monthly rotational grazing was found to provide the optimum conditions for the maximum yield of starch equivalent from pastures. Close-grazing at monthly intervals had the further advantage over more drastic systems of conferring on the herbage a " balance " which rendered it more suited to form the sole diet of farm animals. Lengthening of the interval of unchecked growth from a month to five weeks showed a slight falling off in nutritive value, though the actual yield of starch equivalent and digestible protein may suffer no depression. The following table shows that in Woodman's 1925-26 experiments, though the dry matter produced under hay management was about double that produced by pasture, yet in terms of starch equivalent the yield was approximately the same, while the pasture crop produced considerably more protein per acre:—

	lb. per acre.	
	Pasture.	Meadow.
Dry matter	3667·0	7990·0
Starch equivalent	2532.0	2880.0
Digestible protein	754·0	465·0

Ross et al (1927, 1931) tested the digestibility of Teff and Lucerne hay for sheep and cattle. Whilst the values for these mature materials were very much lower than those for the pasture cuts in Woodman's work, they did not differ markedly from data for material of similar composition elsewhere.

That the intensive rotational system of grassland management holds out definite possibilities in certain regions of the Union has been indicated by some recent experiments. Taylor (1931) kept two Friesland cows from October to May on two-thirds of a morgen of fertilised Kikuyu pasture. The plot was divided into three camps and the grazing rotated on these for the period mentioned. No supplementary feed was given and the production of milk was more than 2,000 gallons per morgen. Analysis of the herbage at intervals during the trial showed the following composition for the dry matter:

Crude protein	19·0 — 28·0 per cent.
CaO	0·33 — 0·53 per cent.
P_2O_5	0·83 — 1·07 per cent.

Moses (1934) presented data which indicated that under normal conditions in the tropical coastal belt of Natal the carrying capacity of a fertilised Kikuyu pasture was in the neighbourhood of 1½ head of mature cattle per acre. Cutting the herbage within enclosed quadrats at the grazing stage produced from 12,000 to 14,000 lb. of dry matter per acre in the course of 12 months. The average crude protein and phosphate (P_2O_5) contents of the dry matter were 14·0 per cent. and 0·80 per cent, respectively.

It is evident then that the main factors influencing the chemical composition of pastures are species of plants, stage of growth of the plants, climatic conditions, and the nature of the soil. Of these factors stage of growth appears to be the most important, in that the percentage of nitrogen, phosphorus, potassium and to a lesser extent calcium and magnesium in the dry matter of the plants falls rapidly from the early tillering stage to maturity. Furthermore, the yield of dry matter is generally highest when the herbage is allowed to reach the mature stage of growth before being harvested, and, finally, increasing maturity depresses the digestibility of the herbage.

Section A.—The Influence of Frequency of Cutting on the Yield and Chemical Composition of Grasses.

III. PLAN OF THE INVESTIGATION.

In February, 1934, an area of natural veld on flat open country about a mile from the Onderstepoort Laboratories was ploughed, the existing vegetation removed and the soil cultivated to a depth of about 9 inches.

The soil profile has been described as follows:

Horizon A_1—About 9 inches deep, consisting of loose crumb structure, reddish brown, light sandy loam of fine texture on the surface and gradually becoming firmer lower down.

Horizon A_2—About 8 inches deep, more compact than A_1, but also of fine sandy appearance, brown in colour.

Horizon B—About 8 inches deep, yellowish-grey in colour, firm and sticky (with moisture) but containing still a high percentage of sand.

Horizon C—Partially weathered and decomposed gravel derived probably from Norite.

The loss on ignition was found to be 4·8 per cent. for horizon A_1, while chemical analysis showed it to contain 0·00025 per cent. available phosphate (P_2O_5), 0·111 per cent. available lime (CaO) and 0·073 per cent total nitrogen.

For the estimation of the available lime and phosphate an extract was prepared by shaking the air-dry soil with one per cent. citric acid in the ratio of 1 : 10 in a suitable container for one hour, allowing the mixture to stand overnight, and shaking it for a further one hour before filtering. Calcium and phosphorus were determined on this extract by the usual methods.

A total of 25 plots, each (24 x 17) square feet was laid out on an area of approximately (60 x 40) square yards in such a manner that each plot was bounded by a path, 3 feet wide, running between plots as indicated on the accompanying chart.

Five species of indigenous grasses, each replicated five times, were planted on the plots in a Latin Square arrangement. The species selected were *Chloris gayana* (Rhodes grass), *Panicum maximum* (hairy Buffelsgras), *Cenchrus ciliaris* (Buffelsgras), *Digitaria Pentzii, Pretoria small*, and *Setaria Lindenbergiana*.

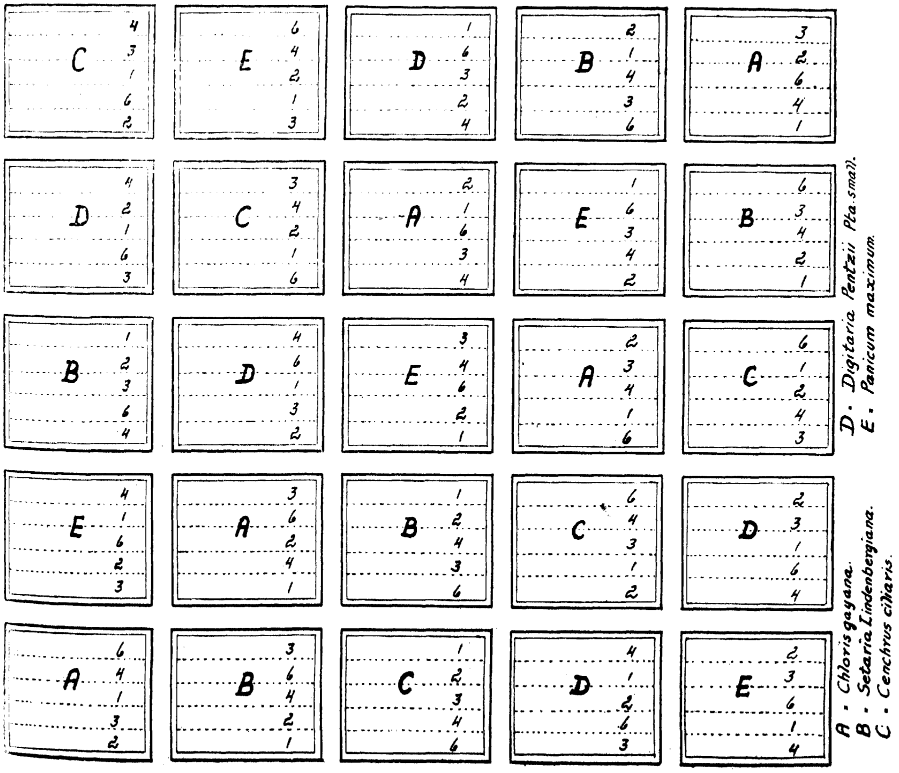
The *Chloris* and *Cenchrus species* were obtained from the Prinshof Pasture Experiment Station, Pretoria, and from Pienaar's River in the Pretoria district, respectively. The *Setaria* species came from Pretoria North while the remaining two grasses were collected locally.

The grasses were propagated from roots and the method of planting was briefly as follows: The aerial portions of the plants which were dug up in such a manner that most of the roots remained intact, were cut off leaving about 4 inches of the basal internodes of the tillers to the roots. The stools were then split up into smaller units of a few tillers with roots each and these planted in rows 12 inches apart, so that only the root portions were underground and in intimate contact with the soil. Each plot was watered after being planted.

Owing to the advanced stage in the season when the grasses were planted and in spite of frequent watering during March and April very little growth took place before winter set in. At the commencement of the following growing season it was observed that with the exception of Rhodes grass and the *Digitaria species* the majority of plants failed to grow. It was consequently decided to utilise the whole of the 1934-35 season for the purpose of establishing uniform stands on all the plots. Due to the growth habit of the *Chloris* and *Digitaria species*, both grasses being of the stoloniferous type sending out runners which root at the nodes and so form new plants, this ideal was realised by the end of the season in the case of the two species mentioned without further attention except occasional watering and weeding. On the other hand much attention had to be devoted to the establishment of the three bunch grasses, especially the *Setaria* and *Panicum species*, in the way of replanting. Where plants failed to grow new plants were introduced during October. Several of these plants again failed to take root and had to be replanted in December and January. Through these efforts fairly uniform stands of growing stools were obtained, but owing to the difference in time of planting a number of stools were inferior in development to the majority which commenced growing during the previous season and early in the 1934-35 season.

Plan of experimental area showing layout of plots.

[*A, B, C, D, and E represent grass species*
1, 2, 3, 4, and 6 represent cutting treatments]



Nevertheless, in April, 1935, the grasses on all plots were cut short. A certain amount of growth took place following this cut. The investigation commenced when on the 25th October, 1935, the remaining dry matter from growth of the previous season was removed by cutting to about 2—4 inches from the ground. Each of the 25 main plots was pegged to a size of (22 × 15) square feet leaving a border of 12 inches wide all round. Main plots were again sub-divided into five equal sub-plots measuring (22 × 3) square feet. A different treatment was allocated at random to each sub-plot within a main plot so that there were five replications for each treatment. A restriction was, however, applied to the randomisation of treatments within plots. Reference to the chart reveals this restriction to be that with regard to rows a treatment occurs once only in the same position within a main plot.

The experiment covered two successive growing seasons and the treatments applied were:—

(1) Cutting the herbage at monthly intervals,

(2) Cutting the herbage at 2-monthly intervals,

(3) Cutting the herbage at 3-monthly intervals,

(4) Cutting the herbage after 4 months' growth followed by an aftermath cut of 2 months' growth, and

(5) Cutting the herbage once only towards the end of the growing season, usually a 6-monthly cut.

The objects of the investigation were to study the effect of the treatments severally on the yield, chemical composition and digestibility of the grasses. Nitrogen, phosphorus, and calcium balances were, however, also determined.

IV. METEOROLOGICAL DATA.

The main features in the weather conditions for the period of the experiment are given in table I. For the purpose of comparison the average monthly rainfall from January to Dcember for the last 25 years is included in the table. It will be seen later that for the two seasons covering the experiment the plots were harvested in each case for the last time early in April. Consequently, the rainfall which could have influenced the composition and yield of the herbage obtained was that recorded for the period September to the end of March. Comparing the rainfall during the period mentioned for the two seasons of the experiment with the average, it is found that the total for both seasons is above the normal. The figures are 27·45 inches and 29·58 inches for the 1935-36 and the 1936-37 seasons, respectively, while the average total for the last 25 years is only 24·95 inches. On the other hand, a glance at figure 1 giving the distribution of the rainfall as monthly totals for the seasons of the experiment reveals several deviations from the normal. Up to January the 1935-36 season was abnormally dry, while the 1936-37 season approximated more closely to normal conditions except for a dry spell in December. During both seasons more rain fell after January than the amount normally registered. However, the

Rainfall (ly total rainfall for the last 25 years included.

Day of Month.	November.	December.	January. 1937.	February.	March.	April.
1	—	—	—	—	—	—
2	—	—	0·32	—	—	—
3	—	—	0·75	0·05	—	—
4	—	—	0·22	0·15	—	—
5	0·08	—	—	0·42	0·04	—
6	—	—	1·14	—	—	0·80
7	—	—	—	0·21	0·03	0·14
8	1·45	—	0·35	0·09	—	—
9	0·26	0·10	—	4·26	—	—
10	0·85	0·04	—	3·89	—	—
11	0·64	0·11	—	0·02	—	—
12	0·26	0·18	—	0·66	—	0·44
13	—	0·10	—	—	—	0·01
14	—	—	0·03	0·72	—	—
15	0·22	0·33	0·04	0·03	—	—
16	—	0·32	—	0·06	0·03	—
17	0·18	—	—	0·06	0·04	—
18	0·71	—	—	—	—	—
19	0·03	—	—	—	—	—
20	0·27	—	0·03	0·02	—	0·03
21	0·03	—	—	—	0·56	—
22	0·34	—	—	—	—	—
23	0·33	—	—	—	—	—
24	0·83	—	0·17	—	—	—
25	—	0·05	0·05	—	0·18	—
26	—	—	0·15	—	—	0·77
27	—	—	0·81	—	—	—
28	—	—	0·30	—	0·16	—
29	0·06	1·46	0·15	—	0·34	—
30	—	0·68	—	—	—	—
31	—	—	—	—	—	—
TOTAL	6·54	3·37	4·51	10·64	1·38	2·20
Average monthly tot for last 25 years	5·33	4·79	4·87	3·84	3·39	0·97
Average Maximum ten perature °F.	82·2	88·0	85·0	84·2	85·8	82·2
Average minimum ten perature °F.	54·5	55·5	59·5	59·4	53·9	45·8

distribution of the rainfall for the latter half of the 1936-37 season was such that plant growth did not receive the maximum benefit from it. Early in February heavy downpours were experienced and this was followed by dry and hot weather intermittently relieved by light ineffective showers. Dry spells during both seasons were accompanied by high maximum temperatures (see fig. 1). The variations in the average monthly minimum temperatures did not show marked differences during the two seasons.

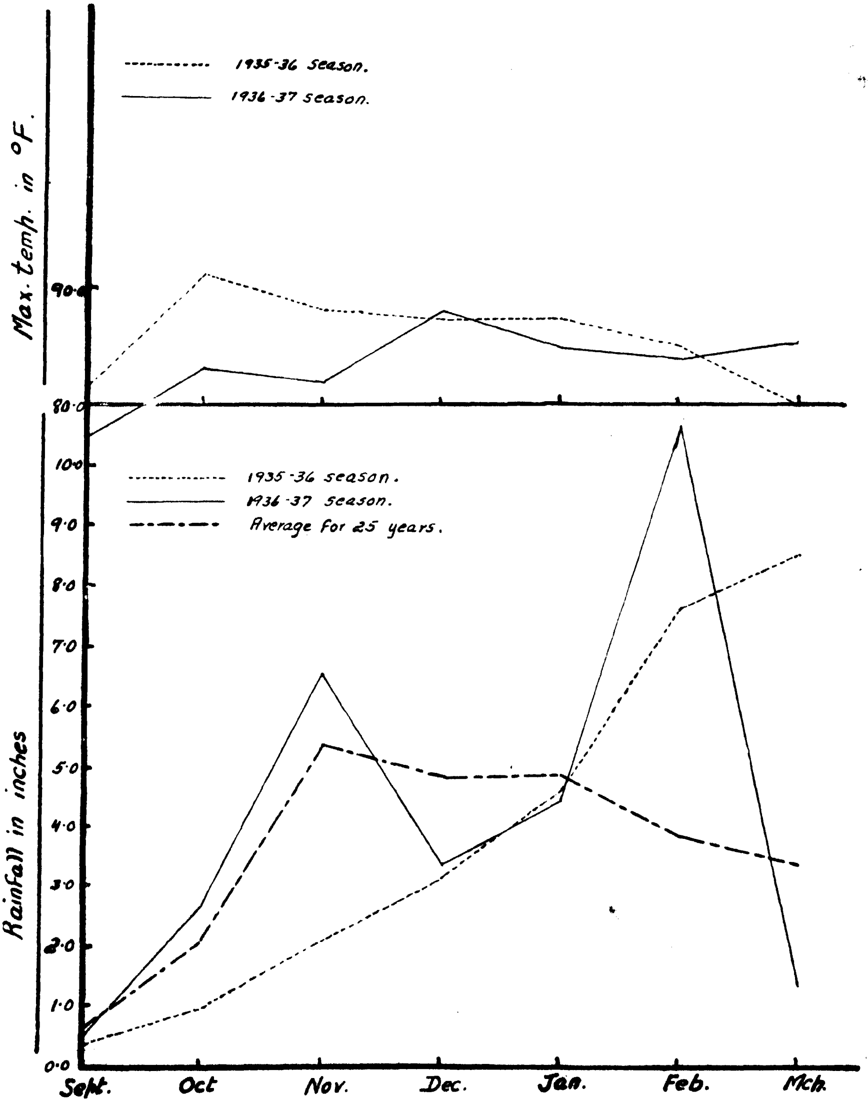


Fig. 1.—Showing monthly rainfall and average maximum temperatures for the seasons 1935-36 and 1936-37.

V. TECHNIQUE.

(1) Cutting and Drying of Herbage.

While it was intended to cut the herbage according to the scheme given under " plan of the investigation ", weather conditions did not permit of this plan being strictly adhered to. Thus, growth was so poor during the first half of the 1935-36 season that the first monthly cuts could not be made before the beginning of January. Until early in April, the concluding date for cutting operations for the season, four monthly cuts and two 2-monthly cuts were obtained. There was one 4-monthly cut, while those portions of the plots which should have been cut after 6 months were harvested at the same time as all other portions early in April, and considered as 4-monthly cuts. The herbage obtained under the 3-monthly system of cutting for the season was made up of a 3-monthly cut on the 3rd March, and another cut only a month afterwards on the 6th April. This was unfortunate since the 3-monthly treatment was, no doubt detrimentally affected by this monthly cut when compared with other more lenient systems.

Reporting on the trend of organic food reserves in Alfalfa roots as affected by cutting practices, Granfield (1935) found that frequent cutting as in pre-flowering stage appeared to result in lower carbohydrate and nitrogen contents of the roots when winter arrived, and conversely with infrequent cutting. Also, the amount of growth which took place after the last regular cutting had a material bearing on the reserves stored in the roots before winter. Permitting the aftermath to remain during the winter, instead of removing it, appeared to result in more vigorous growth during the following spring.

In view of these observations by Granfield the present experiment was designed to have the last cuts as early as possible in April and to allow the small amount of growth taking place before winter set in to remain on the plots. At the same time, the deviations from the general plan of the investigation, exemplified, for instance, by the application of monthly cuts to portions of the plots to which a 3-monthly cutting treatment has been allocated, were made necessary by the circumstance that the investigation aimed at comparing the yield of dry matter under five different treatments and the period of growth for all systems had consequently to be of the same duration: the final cuts for each treatment had to be on the same date.

During the 1936-37 season cutting was commenced with somewhat earlier, the monthly and 2-monthly portions of all plots being cut for the first time on the 15th December. After this date cuts were made according to plan except that no monthly cuts were made in March owing to poor growth.

Cutting was done by hand with a sickle to about 2-4 inches from the ground, depending upon the growth habit of the species. Thus the stoloniferous species, *Chloris* and *Digitaria*, were cut shorter than the three bunch grasses. The material was sampled for dry matter determination immediately after being cut and then spread open on grain bags on the ground to dry in the sun until about 4 p.m. in the afternoon. Small samples were usually found to be almost dry by this time if cut by 10 a.m. However, all samples were collected in hessian bags in the afternoon and removed to a corrugated iron building near the laboratory, where they were hung up to dry in the bags. When air-dry the material was weighed and the figures so obtained were used to compare the yield from the different species and treatments.

It is realised that these figures do not represent the true yield of dry matter as cut, since it has been reported (Kirsch, 1933) that loss of dry matter, presumably through respiration, occurs with even the best known methods of hay-making. Whereas dry matter has been determined on the herbage as cut the best procedure would have been to weigh the fresh herbage and then calculate the weight of dry matter. No facilities were, however, available on the site of the experimental plots so that the only alternative was to weigh the air-dry material. Whilst the method employed does not give absolute values for yield of dry matter it does serve to show up the relative yielding capacities of species and treatments.

It may be added that the material inside the bags was turned over occasionally so that no " heating " of the grass occurred.

(2) Chemical Methods.

For the determination of dry matter about 250-300 gm. of freshly cut herbage was introduced into an ordinary canned-fruit flask and the lid tightly screwed on. The flask with its contents was then taken directly to the laboratory about a mile away and weighed. The herbage was transferred to a small canvas bag and dried to constant weight at 100° C.

For the purpose of determining chemical composition the material from the five replications of any one species and treatment was treated as a single sample. Thus, the air-dry material obtained from the five replications of the monthly cuts of Rhodes grass in January, for instance, was mixed after weighing and a representative sample of about 100 grams taken. This was finely ground, bottled, and put away for chemical analysis.

Crude protein, crude fibre, and ether-soluble extract were determined by the usual methods employed by agricultural workers. Total ash, silica-free ash and the extract for the determination of calcium, magnesium, potassium and sodium were obtained by a method described by Louw (1934). A seperate extract was prepared for the determination of phosphorus and chlorine according to the directions given by Malan and van der Lingen (1931). The essential difference between the methods for preparing the two extracts was the addition of calcium acetate solution to the material before incineration in the case of the phosphorus-chlorine extract. All inorganic constituents, excepting chlorine, were determined by micro-methods. The following are brief deails of the principles on which the methods employed are based :—

(i) *Phosphorus.*—Methods of Bell and Doisy (1920) and Deniges (1920), adapted for the determination of phosphorus in grasses by Malan and van der Lingen (1931).—Principle—Molybdate added to test solution. Phosphomolybdate is formed. This is reduced by hydroquinone and sodium sulphite or by stannous chloride to form a blue compound which is estimated colorimetrically.

(ii) *Calcium.*—Method of Clark and Collip (1935). Principle—Oxalate added to test solution. Calcium oxalate is formed and this is titrated with a 0·01 N potassium permanganate solution.

(iii) *Magnesium.*—Method of Briggs (1924) modified by Malan and Van der Lingen (1931) and Holzapfel (1934). Principle—The calcium present is precipitated as oxalate and subsequently the magnesium as magnesium ammonium phosphate after which the phosphorus is dtermined colorimetrically.

(iv) *Potassium.*—Method of Kramer and Tisdall (1921) adapted to grass analysis by Malan and van der Lingen (1931) and modified by Hubbard (1933). Principle— Potassium is precipitated under standarised conditions as potassium sodium cobalti nitrite which is titrated with 0·01 N potassium permanganate solution.

(v) *Sodium.*—Method of Barrenscheen (1927) modified by Louw (1933). Principle—Sodium is precipitated as the triple acetate, Uranyl-zinc-sodium acetate. Potassium ferro cyanide is added to the triple acetate in solution. A brown colour is formed, the intensity of which is proportional to the amount of uranium present.

(vi) *Chlorine.*—Volhard's method for the detection of halides was adapted by Malan and van der Lingen for the determination of chlorine in grasses. Principle—Chloride is precipitated as silver chloride by the addition of silver nitrate to the test solution and the excess silver nitrate titrated with potassium sulpho-cyanide.

VI. RESULTS.

(1)The Influence of Frequency of Cutting on the Yield of dry Matter.

The aggregate seasonal yields of air-dry herbage for individual cutting treatments, species, and replications for the two seasons of the experiment are given in tables 2 and 4. The arrangement of the data within the tables is similar to the lay-out of the plots (see chart), except that the figures giving the yields of air-dry herbage for treatments have been re-arranged within rows in order to facilitate the drawing up of the tables. Row totals and column totals are included in the tables as these figures are made use of in the method employed for the statistical analysis of the data. Two additional tables are given for the purpose of applying this analysis. These are tables 3 and 5 giving the grand ~~seasonal~~ totals for air-dry material for species and cutting treatments.

The following is a statistical analysis of the yield data made under the direction of Dr. A. Laurence of the Division of Economics and Markets, Union Department of Agriculture.

(*a*) 1935-36 *Season.*

Crude Calculations from Tables 2 *and* 3.

Total sum of squares of deviations for all sub-plots = (Sum of squares for sub-plots) — $\frac{(\text{Grand total})^2}{125}$
= 350,192,293 — $\frac{(198,301)^2}{125}$
= 350,192,293 — 314,586,293
= *35,606,000*

Total sum of squares of deviations for plots = $^1/_5$ (Sum of squares for plots) — 314,586,293
= $^1/_5$ (1,710,795,019) — ,,
= 342,159,004 — ,,
= *27,572,711.*

Sum of squares for rows = $^1/_{25}$ (Sum of squares for row totals) — 314,586,293
= $^1/_{25}$ (7,889,521,591) — ,,
= 315,580,864 — ,,
= *994,571.*

Sum of squares for columns = $^1/_{25}$ (Sum of squares for column totals) — 314,586,293
= $^1/_{25}$ (7, 994,996,067) — ,,
= 317,799,843 — ,,
= *3,213,550.*

Sum of squares for varieties = $^1/_{25}$ (Sum of squares for variety totals) — 314,586,293
= $^1/_{25}$ (8, 334,076,755 — ,,
= 333,363,070 — ,,
= *18,776,777.*

Total sum of squares = $^1/_5$ (Sum of squares of 25 totals in table 3) — 314,586,293
= $^1/_5$ (1,689,171,211) — ,,
= 337,834,242 — ,,
= *23,247,949.*

Sum of squares for cuts = $^1/_{25}$ (Sum of squares for cut totals) — 314,586,293
= $^1/_{25}$ (7,933,707,285) — ,,
= 317,348,291 — ,,
= *2,761,998.*

Sum of squares for inter-action (cuts *X* varieties) = (Total sum of squares) — (sum of squares for varieties + sum of squares for cuts)
= 23,247,949 — (18,776,777 + 2,761,998)
= *1,709,174.*

TABLE 2.

Yields of Air-dry grass in grams per 66·0 *square feet.*

1935-36 *Season.*

Cutting Intervals.	C.	E.	D.	B.	A.	Total.	Row Totals.
1 month	1,037	1,382	953	1,162	1,418	6,452	
2 months	1,523	1,661	761	1,963	1,205	7,113	
3 months	1,444	1,979	951	2,055	1,426	7,855	37,875
4 months	1,676	1,765	1,070	2,097	1,745	8,353	
6 months	1,470	1,658	869	2,723	1,382	8,102	
TOTAL	7,150	8,445	4,604	10,500	7,176	37,875	
	D.	C.	A.	E.	B.		
1 month	1,043	1,011	851	2,256	2,240	7,401	
2 months	1,043	1,190	1,194	2,808	2,273	8,508	
3 months	1,231	1,499	931	2,434	2,314	8,409	42,764
4 months	1,243	1,230	1,265	2,940	2,285	8,963	
6 months	1,185	1,743	1,117	2,728	2,710	9,483	
TOTAL	5,745	6,673	5,358	13,166	11,822	42,764	
	B.	D.	E.	A.	C.		
1 month	2,138	882	1,328	853	1,195	6,395	
2 months	2,046	1,029	1,276	2,198	1,254	7,803	
3 months	2,079	935	1,431	1,422	1,632	7,499	38,015
4 months	2,265	1,365	1,375	1,420	1,552	7,977	
6 months	2,235	990	1,547	1,170	2,398	8,340	
TOTAL	10,763	5,201	6,957	7,063	8,031	38,015	
	E.	A.	B.	C.	D.		
1 month	1,757	1,420	1,637	938	887	6,639	
2 months	1,896	1,282	1,379	1,210	1,127	6,894	
3 months	1,916	1,567	1,911	1,229	964	7,587	37,669
4 months	2,200	1,655	1,496	1,157	1,734	8,242	
6 months	2,078	1,712	1,922	1,322	1,273	8,307	
TOTAL	9,847	7,636	8,345	5,856	5,985	37,669	
	A.	B.	C.	D.	E.		
1 month	1,254	1,848	918	933	1,388	6,341	
2 months	2,212	2,655	1,029	1,012	2,137	9,045	
3 months	1,371	2,044	1,205	1,095	1,950	7,665	41,978
4 months	1,495	2,686	1,379	1,152	3,104	9,816	
6 months	1,715	1,964	2,380	970	2,082	9,111	
TOTAL	8,047	11,197	6,911	5,162	10,661	41,978	
COLUMN TOTALS	41,552	39,152	32,175	41,747	43,675	—	198,301

TABLE 3.

Yields of Air-dry grass in grams per 330 square feet, showing varietal, cutting, and interaction effects.

1935-36 *Season.*

(Means in brackets).

Cutting Intervals.	A.	B.	C.	D.	E.	Total.
1 month..........	5,796 (1,159·2)	9,525 (1,905·0)	5,099 (1,019·8)	4,698 (939·6)	8,111 (1,622·2)	33,229
2 months.........	8,091 (1,618·2)	10,316 (2,063·2)	6,206 (1,241·2)	4,972 (954·4)	9,778 (1,955·6)	39,363
3 months.........	6,717 (1,343·4)	10,403 (2,080·6)	7,009 (1,401·8)	5,176 (1,035·2)	9,710 (1,942·0)	39,015
4 months.........	7,580 (1,516·0)	10,829 (2,165·8)	6,994 (1,398·8)	6,564 (1,312·8)	11,384 (2,276·8)	43,351
6 months.........	7,096 (1,419·2)	11,554 (2,310·8)	9,313 (1,862·6)	5,287 (1,057·4)	10,093 (2,018·6)	43,343
TOTAL......	35,280	52,627	34,621	26,697	49,076	198,301

Analysis of Variance.

	Degrees of freedom.	Sum of squares.	Mean Square.	Standard deviation.	$\log_e$ S.D.	z.
Rows.............	4	994,571	—	—	—	—
Columns..........	4	3,213,550	—	—	—	—
Varieties..........	4	18,776,777	4,694,194	2,166·6	7·68094	1·25395
Error (*a*).........	12	4,587,813	382,318	618·3	6·42699	—
Total plots........	24	27,572,711	—	—	—	—
Cuts..............	4	2,761,998	690,499·5	831·0	6·72264	1·37077
Interaction........	16	1,709,174	106,823	326·8	5·78937	0·43750
Error (*b*).........	*80*	*3,562,117*	44,526·5	211·0	5·35187	—
Total sub-plots....	124	35,606,000	—	—	—	—

From Fisher's (1932) tables giving the z values for different degrees of significance we have that for $n_1=4$ and $n_2=12$, $z=0{\cdot}5907$ at P = ·05, and $z=0{\cdot}8443$ at P = ·01; for $n_1=4$ and $n_2=60(80)$, $z=0{\cdot}4632$ at P = ·05, and $z=0{\cdot}6472$ at P = ·01, and for $n_1=16$ and $n_2=80$, $z=0{\cdot}2855$ at P = ·05, and $z=0{\cdot}3975$ at P = ·01. Hence from a comparison of the observed and the theoretical values for z it is concluded that varieties are significantly different, cuts are significantly different, and interaction (variety × cuts) is significantly different, all at P = ·01. It is therefore permissible to proceed to the t-test to discover where this significance lies.

(i) *Significant differences between varieties.*

Variety.		Totals.	Means.
Chloris gayana	(A)	35,280	1,411·2
Setaria Lindenbergiana	(B)	52,627	2,105·1
Cenchrus ciliaris	(C)	34,621	1,384·8
Digitaria Pentzii Pretoria Small	(D)	26,697	1,067·9
Panicum maximum	(E)	49,076	1,963·0

The standard error of a single plot is 618·3, and of the mean of 25 plots is $\frac{618\cdot3}{\sqrt{25}}$. The standard error of the difference between two

$$\text{means} = \frac{618\cdot3 \times \sqrt{2}}{\sqrt{25}}$$
$$=174\cdot9.$$

For $n=12$, the degrees of freedom on which the estimate of error is based, $t=2\cdot179$ at $P=\cdot05$, and $t=3\cdot055$ at $P=\cdot01$ (Fisher's tables). Hence for significance at $P=\cdot05$, the difference must be $=(2\cdot179 \times 174\cdot9)=381\cdot1$, and at $P=\cdot01$, the difference must be $(3\cdot055 \times 174\cdot9)=534\cdot3$. Whence it may be concluded that while there is no significant difference between the *Setaria* and *Panicum* species, both these species yielded significantly more dry matter than the *Chloris*, *Digitaria*, and *Cenchrus* varieties, the odds being over 100 : 1 in favour of this significant variety effect. The three last-mentioned varieties are insignificantly different.

(ii) *Significance of differences between cuts.*

Cutting intervals.	Totals.	Means.
4 months	43,351	1,734·0
6 months	43,343	1,733·7
2 months	39,363	1,574·5
3 months	39,015	1,560·6
1 month	33,229	1,329·2

The standard error of a single sub-plot $=211\cdot0$, and the standard error of the difference between means of 25 sub-plots

$$=\frac{211\cdot0 \times \sqrt{2}}{\sqrt{25}}$$
$$=59\cdot7.$$

For $n=80$ (30), $t=2\cdot042$ at $P=\cdot05$, and
$t=2\cdot750$ at $P=\cdot01$.

For significance at $P=\cdot05$, difference must, therefore be
$=(2\cdot042 \times 59\cdot7)$
$=121\cdot9$, and

for significance at $P=\cdot01$, difference must be
$=2\cdot750 \times 59\cdot7$
$=164\cdot2$.

Whence the statistically significant elements in the results may be summarised as follows: Cutting at 2, 3, 4, and 6 monthly intervals yielded significantly more dry matter than cutting at monthly intervals, the odds being over 100 : 1 in favour of this cutting effect. Also the 4 and 6 monthly cutting systems are better than the 3-monthly system at $P = \cdot 01$, and the 2-monthly system at $P = \cdot 05$. Other differences are not significant.

(iii) *The significance of interaction.*

This significance means that we are premitted to seek the significant differences within the body of table 3, where the means are given in brackets. These variety means are tabulated below in decreasing order of magnitude. The figures preceding the means refer to the cutting interval.

Variety A.		Variety B.		Variety C.		Variety D.		Variety E.	
2,	1,618·2	6,	2,310·8	6,	1,862·6	4,	1,312·8	4,	4,276·8
4,	1,516·0	4,	2,165·8	3,	1,401·8	6,	1,057·4	6,	2,018·6
6,	1,419·2	3,	2,080·6	4,	1,398·8	3,	1,035·2	2,	1,955·6
3,	1,343·4	2,	2,063·2	2,	1,241·2	2,	954·4	3,	1,942·0
1,	1,159·2	1,	1,905·0	1,	1,019·8	1,	939·6	1,	1,622·2

The standard error of a single sub-plot $= 211 \cdot 0$, and hence the standard error of the difference between means of 5

$$= \frac{211 \cdot 0 \times \sqrt{2}}{\sqrt{5}}$$

$$= 133 \cdot 4.$$

For $n = 80$ (30), $t = 2 \cdot 042$ at $P = \cdot 05$, and
$t = 2 \cdot 750$ at $P = \cdot 01$.

For significance at $P = \cdot 05$, difference must, therefore, be
$= (2 \cdot 042 \times 133 \cdot 4)$
$= 272 \cdot 4$, and

for significance at $P = \cdot 01$, difference must be
$= (2 \cdot 750 \times 133 \cdot 4)$
$= 366 \cdot 9$.

Whence for *Chloris gayana*, a 2-monthly cutting rotation yields significantly more dry matter than a 3-monthly cutting at $P = \cdot 05$, and yields significantly more dry matter than a monthly cutting system at $P = \cdot 01$; also, a 4-monthly system is significantly better than a monthly system at $P = \cdot 05$.

For *Setaria Lindenbergiana*, significantly more dry matter will be obtained by cutting after an interval of 6 months than by cutting the herbage every month, the odds being over 100 to 1 in favour of this cutting effect.

For *Cenchrus ciliaris*, cutting after an interval of 6 months is significantly better than cutting after intervals of either 1, 2, 3, or 4 months at $P = \cdot 01$. The 3- and 4-monthly cutting systems are similarly significantly superior to the monthly system of cutting.

For the *Digitaria species*, and the *Panicum* variety a rest period of 4 months is significantly better than a rest period of only one month at $P = \cdot 01$, and significantly better than rest periods of 2 and 3 months at $P = \cdot 05$. Also, in the case of the *Panicum* variety, significantly more dry matter is obtained by allowing the herbage to grow undisturbed for six months before it is harvested than by cutting it at monthly intervals, the odds being more than 100 : 1 in favour of the six-monthly cutting rotation.

All other differences not mentioned in the summary of conclusions are not significant.

(*b*) *The* 1936-37 *Season.*

The results for the above mentioned season are given in tables 4 and 5. The statistical analysis of these results was executed in a manner similar to what has been done with the data for the 1935-36 season. From the analysis of variance it was found that varieties are significantly different at $P = \cdot 05$, and cuts are significantly different at $P = \cdot 05$. The z value appropriate to interaction and error was, however, observed to be smaller than the theoretical z value from Fisher's tables for $n_1 = 16$ and $n_2 = 80$ at both the one per cent. and the five per cent. points of distribution. The t-test was, therefore, applied only to cuts and varieties. The following is a summary of the conclusions drawn:

(i) *Varieties.*

Both *Panicum maximum* and *Setaria Lindenbergiana* produce significantly more dry matter than either *Cenchrus ciliaris* or the *Digitaria species* at $P = \cdot 05$. *Chloris gayana*, *Panicum maximum*, and *Setaria Lindenbergiana* are insignificantly different in regard to their respective yielding capacities. The same insignificant difference is found between *Cenchrus ciliaris*, *Chloris gayana* and the *Digitaria species.*

(ii) *Cuts.*

Significantly more dry matter is produced by cutting at 6- and 4-monthly intervals than by cutting at 3- and 1-monthly intervals, and a 2-monthly system is decidedly superior to a monthly system for the production of dry herbage, the odds being more than 100 : 1 in favour of the said significant cutting effects. The yields of dry matter under a 2-monthly and a 3-monthly cutting system are significantly different in favour of the former system. On the other hand, the yield of dry matter from cutting at 2-, 4-, and 6-monthly intervals is insignificantly different.

TABLE 4.

Yields of Air-dry grass in grams per 66·0 square feet.
1936-37 *Season.*

Cutting Intervals.	C.	E.	D.	B.	A.	Total.	Row Totals.
1 month............	719	1,149	1,034	1,288	1,648	5,838	34,099
2 months...........	1,028	1,410	1,014	1,872	1,399	6,723	
3 months...........	817	1,650	825	1,297	1,445	6,034	
4 months...........	1,350	1,477	973	2,177	1,772	7,749	
6 months...........	1,107	1,213	1,210	2,643	1,582	7,755	
TOTAL.........	5,021	6,899	5,056	9,277	7,846	34,099	
	D.	C.	A.	E.	B.		
1 month............	896	591	717	2,449	1,930	6,583	42,722
2 months...........	1,172	1,049	1,023	3,040	2,262	8,546	
3 months...........	1,415	890	877	3,150	1,560	7,892	
4 months...........	1,203	1,214	1,201	3,164	2,670	9,452	
6 months...........	1,403	1,562	1,207	3,582	2,495	10,249	
TOTAL.........	6,089	5,306	5,025	15,385	10,917	42,722	
	B.	D.	E.	A.	C.		
1 month............	1,868	800	1,058	658	907	5,291	32,583
2 months...........	1,860	930	1,210	2,118	996	7,114	
3 months...........	1,632	945	1,425	1,328	1,105	6,435	
4 months...........	1,465	900	1,145	1,290	1,381	6,181	
6 months...........	1,872	928	1,310	897	2,555	7,562	
TOTAL.........	8,697	4,503	6,148	6,291	6,944	32,583	
	E.	A.	B.	C.	D.		
1 month............	1,291	1,373	1,032	709	630	5,035	30,247
2 months...........	1,438	1,069	1,184	1,064	1,032	5,787	
3 months...........	1,280	1,315	1,120	771	780	5,266	
4 months...........	2,215	1,563	1,257	950	1,453	7,438	
6 months...........	1,593	1,430	1,663	1,062	973	6,721	
TOTAL.........	7,817	6,750	6,256	4,556	4,868	30,247	
	A.	B.	C.	D.	E.		
1 month............	937	1,722	872	562	914	5,007	34,970
2 months...........	2,297	2,238	940	382	1,656	7,513	
3 months...........	1,315	1,653	924	675	1,580	6,147	
4 months...........	1,527	2,064	1,495	675	2,847	8,608	
6 months...........	1,245	1,682	2,352	573	1,843	7,695	
TOTAL.........	7,321	9,359	6,583	2,867	8,840	34,970	
COLUMN TOTALS	34,945	32,817	29,068	38,376	39,415	—	174,621

Summarising the main features in the results of the two seasons of the experiment with reference to the statistically significant elements it is found that of the grass species studied *Setaria Lindenbergiana* and *Panicum maximum* rank first as producers of dry matter. In regard to cutting treatment the most outstanding feature is the depressing effect on the yield of dry matter of a system whereby grasses are cut at monthly intervals during the growing season when compared with more lenient systems of cutting. This effect has been observed for all the species combined as well as for species individually. Of the more lenient cutting treatments the 6- and 4-monthly systems produced on the whole more dry matter than the 2- and 3-monthly systems. The inferior position taken in by the 3-monthly system during the 1936-37 season cannot be accepted as normal without further proof, in view of the fact that a monthly cutting treatment, mentioned earlier in this report, had to be applied at the end of the previous season to suit the requirements of the experiment. Furthermore, the evidence from the results of species combined in the 1936-37 season and from the results of individual species for the previous season suggests that the amount of dry matter obtainable under a 2-monthly system of cutting may not differ materially from that from one cut at the end of the season or two cuts as represented by a 4-monthly cut in February followed by an aftermath cut two months afterwards.

Table 5.

Yields of Air-dry grass in grams per 330·0 *square feet, showing varietal, cutting, and interaction effects.*

1936-37 *Season.*

Cutting Intervals.	A.	B.	C.	D.	E.	Total.
1 month..........	5,333	7,840	3,798	3,922	6,861	27,754
2 months.........	7,906	9,416	5,077	4,530	8,754	35,683
3 months.........	6,280	7,262	4,507	4,640	9,085	31,774
4 months.........	7,353	9,633	6,390	5,204	10,848	39,428
6 months.........	6,361	10,355	8,638	5,087	9,541	39,982
TOTAL......	33,233	44,506	28,410	23,383	45,089	174,621

To conclude these remarks it should be pointed out that the results obtained are probably valid only for the conditions of soil and climate under which the grasses were grown.

(2) The Influence of Climate on the Yield of Dry Matter.

Among the many factors which influence crop production rainfall is the major determinant of yield, especially in regions where the reliability of effective rains is not high. It is, in addition, a common experience that the amount of annual rainfall is not as important as the quantities falling at definite periods during the growing season. Unfortunately, it appears that little or nothing has been done in the summer rainfall area of the Union to ascertain during which period or periods of the growing season rain has the greatest influence on the production of grass species. Work done by du Toit, et al (1935) and by Taylor (1929, 1932) seems to indicate that different species of grasses have different periods of optimum growth under normal conditions of rainfall. In a region of summer-rainfall the atmospheric factors of temperature, humidity, and wind velocity may have a profound influence on the effectiveness of rainfall by governing the rate of evaporation and transpiration.

The seasonal production of the five species studied is given in Tables 6 and 7 for the seasons 1935-36 and 1936-37, respectively. The figures represent the total amount of air-dry herbage obtained from five replications by cutting at monthly intervals in the course of the growing season. Since the general trend in regard to seasonal production is the same for all species the means for all species have been used to illustrate the effect of rainfall on the seasonal production in graphical form. Furthermore, the totals given for January, 1936, and for December, 1936, and again for April, 1937, do not represent growth during one month only and cannot therefore represent a single point in a graph describing true monthly totals. To overcome this difficulty the total for January, 1936, has been split up into three equal portions to represent monthly cuts for November, December and January. Although very little, nevertheless, growth did commence during the 1935-36 season in October and continued intermittently to the date on which the herbage was cut for the first time in January. The splitting up of the cumulative growth in January into three portions as indicated is thus justfied under the circumstances and will give a truer picture of the effect of rainfall on growth. For similar reasons the totals for December, 1936, and April, 1937, have been split up to represent two points on the graph.

TABLE 6.

Showing seasonal production of dry matter in grams.
1935-36 *Season.*

Species.	January.	February.	March.	April.
Chloris gayana	1,507	1,315	1,713	1,261
Setaria Lindenbergiana	2,797	1,787	3,376	1,565
Cenchrus ciliaris	1,384	1,230	1,638	847
Digitaria Pentzii Pta. Sm.	1,254	1,154	1,478	812
Panicum maximum	1,823	2,308	2,841	1,139
TOTAL	8,765	7,794	11,046	5,624
Mean	1,753	1,559	2,209	1,125

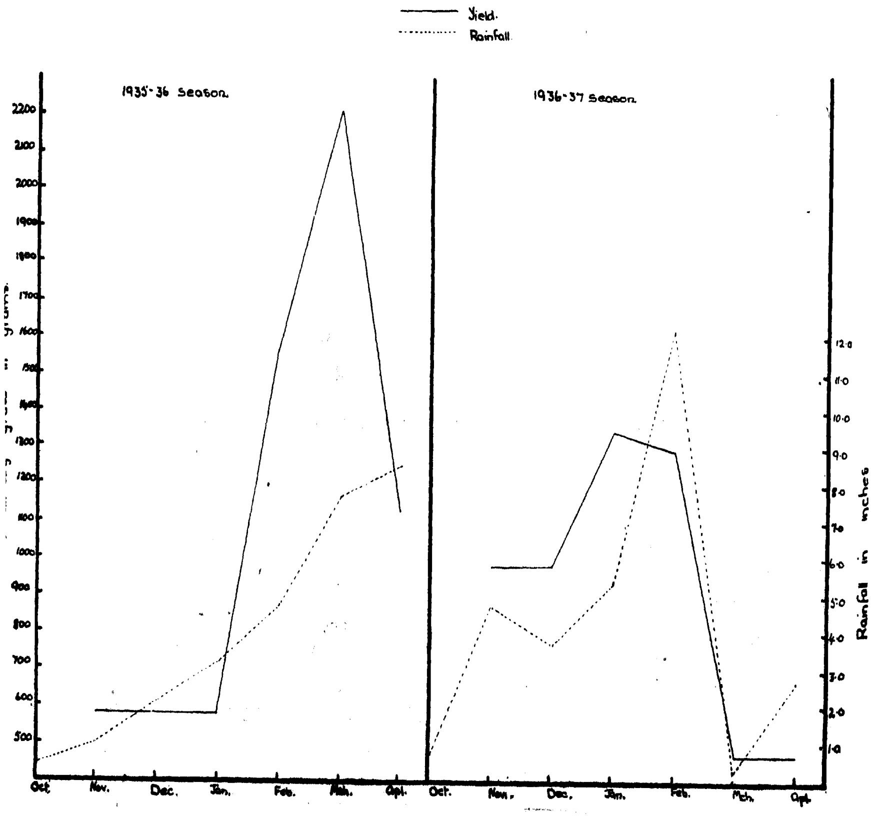
TABLE 7.

Showing seasonal production of dry matter in grams.

1936-37 *Season.*

Species.	December.	January.	February.	April.
Chloris gayana	1,950	1,187	1,153	1,043
Setaria Lindenbergiana	3,163	1,770	1,796	1,111
Cenchrus ciliaris	938	943	855	1,062
Digitaria Pentzii Pta. Sm	1,179	1,211	1,135	397
Panicum maximum	2,611	1,624	1,532	1,094
TOTAL	9,841	6,735	6,471	4,707
Mean	1,968	1,347	1,294	942

Figure 2. Showing effect of rainfall on the seasonal yield of grasses.



The monthly yields of dry herbage for the two seasons are represented in graphical form in conjunction with the monthly rainfall in figure 2. The total rainfall for the month preceding a cut has been made to coincide with the point representing the yield of grass from that particular cut. Generally speaking, the curve describing the yield of dry matter may be considered to vary in a manner similar to that depicting the rainfall for both seasons. In connection with remarks made earlier in this section there are, however, certain features in the graphs which deserve to be mentioned. For the 1935-36 season the period preceding the first cut in January was characterised by a low rainfall associated with high average day temperatures (cf. Fig. 1). During this period only about 26 per cent. (cf. Table 6) of the total growth for the season took place. Good rains during January and February resulted in active growth, the grasses reaching maximum seasonal production with the cut made early in March. In spite of the fact that the best rainfall for the season was recorded for the month following the cut early in March the production of herbage decreased by fully 50 per cent.

Turning now to the data for the 1936-37 season it is found that with climatic conditions approximating the normal about 60 per cent. of the total seasonal production of herbage was obtained with the two cuts in December and January. The yield in February was practically the same as for January but the next two months contributed only about 17 per cent. towards the total for the season. The rainfall during this final period was decidedly low, especially during March, and the average day temperatures were high. At the time of the February cut the soil was over-saturated with moisture as the result of the heavy downpours experienced a few days earlier. Nevertheless, growth was observed to be extremely slow, even while the soil was still wet. The effect of the drastic treatment (monthly cuts) on the vitality of the plants may have been partly responsible for poor growth directly after the February cuts. However, the yield of dry matter under more lenient systems of cutting was also greatly reduced during this final period of the season. For instance, the indices for average seasonal yield of dry matter for species combined for the 2-monthly cutting treatment were 2,439, 3,338, and 1,360 for December, February, and April ,respectively.

In any case, it would appear that, while during seasons of normal rainfall the period of optimum growth is independent of the rainfall, it does depend on rainfall during dry seasons. For the 1935-36 season this period of most active growth occurred at a later date than during the following season. The evidence here produced and from the previous publication (1935) seems to suggest that in the case of the species studied the period of optimum growth is December-January (cf. 1936-37 season and previous results), but that in the event of negligible rain prior to December or January it may occur later in the season (cf. 1935-36 season).

In this connection the results of Trumble and Cornish (1936) are of interest. From a statistical analysis of data pertaining to the influence of rainfall on the yield of natural pasture at the Waite Institute, Australia, a region of winter-rainfall, these workers found the correlation between yield and rainfall to be strongest in the period April to June, which coincides with the early stages of seasonal growth.

(3) THE INFLUENCE OF FREQUENCY OF CUTTING ON CHEMICAL COMPOSITION.

The detailed analyses of the herbage samples obtained under different frequencies of cutting for the whole of the experimental period are given in the appendix by Tables A to I. The last columns in the tables giving a description of the grasses as cut require explanation. These notes aim at giving a brief description with reference to the general appearance and the stage of growth obtained at the time of cutting. Height measurements were not actually made. However, when the grass was estimated to have grown to a height of only 6 inches and less the term " short ", and when to a height of more than 12 inches the term " long " has been used to describe the appearance. No descriptive term was applied for estimated heights between 6 and 12 inches. Furthermore, an attempt has been made to indicate the stage of growth reached by the herbage by using the terms " pre-flowering ", " a few flower-heads appearing ", " flowering ", " with flowerheads ", " seeds falling out ", and " seeds fallen out, inflorescences and some leaves browning ", to indicate the advancing stages of maturity.

In connection with the arrangement of results into separate tables for different cutting treatments it should be pointed out that the cuts of the 6th April in table C are in reality monthly cuts and not 3-monthly cuts; also, the April cuts in table H are 2-monthly and not 4-monthly cuts. Furthermore, since there was no difference between 4- and 6-monthly treatments in the 1935-36 season the analysis given in table D is that for a single final cutting for each species.

From the tables in the appendix tables 8-12 giving the average composition of the dry matter obtained under the different systems of cutting have been compiled. Figures for total ash have been included. These are not reliable owing to the unavoidable inclusion of traces of soil which cannot be removed in the cleaning of the sample. However, the values for silica-free ash form a reliable index of the total minerals present in the herbage.

It is not intended to discuss the chemical composition of the herbage in detail as this has been done for similar data in previous publications by du Toit and his associates. The salient features which have a direct bearing on the present investigation may, however, again be indicated briefly.

(*a*) *Phosphorus.*—While the magnitude of the decrease differs with species the phosphorus content of the herbage diminishes on the whole uniformly from monthly cuts to 6-monthly cuts, the relative amount in the single cut at the end of the season being only about 50 per cent. of that present in the monthly cut herbage. In all cases the figures are low compared with the figures of European investigators but are similar to values obtained previously at Onderstepoort and by Taylor (1922, 1929), and compare favourably with data secured by Australian workers (Richardson et al, 1931, 1932) for grasses at the same stage of growth.

(*b*) *Calcium and Magnesium.*—The percentages of these constituents do not show appreciable variation with stage of growth. With the exception of *Chloris gayana* and *Setaria Lindenbergiana* there appears to be a tendency for these minerals to decrease with decreased frequency of cutting. Petrie (1934) pointed out that the relative amount of calcium declines during adolescence and thereafter remains constant or rises.

(*c*) *Potassium and Chlorine.*—Both these constituents are influenced to a considerable extent by the stage of maturity of the herbage. With few exceptions (cf. K-values in 4-monthly cuts for the 1936-37 season) a fall in the content of potassium and chlorine has been observed with advancing stage of maturity.

(*d*) *Sodium.*—Whilst fluctuating somewhat in some species the general tendency for this element is also to decrease with age in the plant.

(*e*) *Crude protein.*—Generally speaking, the variations due to the influence of cutting treatment in the content of crude protein of the herbage are similar to those for phosphorus, the relative amount falling rapidly with decreased frequency of cutting.

(*f*) *Crude Fibre.*—The crude fibre content of the herbage varies more or less inversely with the crude protein content, the highest percentage of the former constituent corresuonding with the lowest value for the latter.

(*g*) *The Ether-soluble extract* of different species shows different variations with age in the plants. In the case of *Panicum maximum* and *Cenchrus ciliaris* there is a definite drop in the percentgae ether-soluble extract with increasing degree of maturity for both seasons of the experiment. The same tendency is noticeable for the other species studied for the 1935-36 season, but during the following season the values for ether-extract show irregular fluctuations with the different treatments.

(*h*) *Silica-free Ash.*—Since all the mineral elements determined are constituents of the silica-free ash and whereas with few exceptions the relative amounts of these elements decrease with decreasing frequency of cutting it may be expected that the percentage of silica-free ash will vary in a similar manner. A glance at the tables shows this to be the case.

(*i*) *Nitrogen-free extract.*—The percentage content of this constituent in the herbage of varying degree of maturity varies very little and shows no definite tendency. It should, however, be pointed out that the accuracy of the figure for this fraction is dependent upon the accuracy with which the fractions on which its calculation is based have been determined. As previously stated the figure for total ash, one of the relevant fractions, is unreliable owing to the unavoidable inclusion of soil particles in the samples analysed.

TABLE 8.

Average percentage composition of the dry matter of Chloris gayana.

Period of growth.	Season.	P.	Ca.	Mg.	K.	Na.	Cl.	Crude protein.	Crude fibre.	Ether soluble extract.	Total Ash.	N-free extract.	Silica free Ash.	Percentage dry matter of herbage as cut.
1 month............	1935-36	·13	·37	·25	·76	·56	·90	8·3	31·8	2·55	10·52	46·96	4·15	30·7
	1936-37	·12	·43	·29	·77	·38	·67	6·4	32·5	2·27	11·06	47·77	3·75	32·4
2 months............	1935-36	·12	·36	·22	·71	·49	·84	6·4	33·6	2·26	10·29	47·45	3·74	30·8
	1936-37	·11	·39	·23	·75	·32	·65	6·5	31·7	2·63	10·52	48·55	3·25	33·7
3 months............	1935-36	·094	·32	·20	·66	·40	·72	4·8	35·2	2·02	9·28	48·70	3·25	31·5
	1936-37	·088	·42	·25	·55	·31	·64	5·2	32·2	2·46	10·05	50·09	3·10	35·5
4 months............	1935-36	·074	·36	·23	·51	·32	·56	4·4	33·6	2·09	9·86	50·05	2·95	34·1
	1936-37	·077	·37	·23	·64	·27	·55	4·3	32·7	2·14	10·00	50·86	3·09	36·0
6 months............	1936-37	·065	·44	·31	·43	·23	·49	3·9	34·3	2·64	11·25	47·91	3·06	41·0

TABLE 9.

Average percentage composition of the dry matter of Setaria Lindenbergiana.

Period of growth.	Season.	P.	Ca.	Mg.	K.	Na.	Cl.	Crude protein.	Crude fibre.	Ether soluble extract.	Total Ash.	N-free extract.	Silica free Ash.	Percentage dry matter of herbage as cut.
1 month............	1935-36	·16	·39	·41	2·25	·046	·96	10·9	35·7	3·22	11·72	38·46	5·35	31·3
	1936-37	·15	·44	·41	1·58	·27	·60	9·7	35·2	3·35	11·03	40·72	4·68	33·0
2 months............	1935-36	·13	·44	·41	1·58	·043	·77	7·6	37·4	3·01	11·75	40·24	4·49	33·2
	1936-37	·13	·50	·47	1·23	·092	·59	7·6	35·2	3·46	11·59	42·15	4·12	34·4
3 months............	1935-36	·11	·41	·36	1·36	·044	·60	5·9	38·3	3·55	11·31	41·94	3·82	33·6
	1936-37	·09	·50	·42	1·05	·040	·46	5·8	37·3	3·18	11·37	42·35	3·90	39·6
4 months............	1935-36	·088	·39	·32	1·16	·020	·51	5·2	39·3	2·62	11·25	41·63	3·52	37·6
	1936-37	·091	·43	·36	1·05	·036	·46	4·9	39·4	2·81	11·15	41·74	3·67	38·9
6 months............	1936-37	·078	·55	·37	·84	·057	·38	4·8	35·3	3·62	13·10	43·18	3·82	51·7

TABLE 10.

Average percentage composition of the dry matter of Cenchrus ciliaris.

Period of growth.	Season.	P.	Ca.	Mg.	K.	Na.	Cl.	Crude protein.	Crude fibre.	Ether soluble extract.	Total Ash.	N-free extract.	Silica free Ash.	Percentage dry matter of herbage as cut.
1 month.............	1935-36	·17	·34	·42	2·45	·077	·84	11·2	29·4	2·88	15·50	41·02	5·69	27·6
	1936-37	·13	·36	·39	1·63	·28	·56	9·3	30·1	2·79	14·95	42·86	4·58	31·3
2 months............	1935-36	·14	·32	·40	2·15	·065	·66	9·2	33·4	2·39	15·25	39·76	5·19	30·0
	1936-37	·12	·35	·38	1·57	·091	·54	8·6	31·6	2·74	14·82	42·24	4·35	32·2
3 months............	1935-36	·12	·30	·40	1·89	·059	·67	7·1	35·4	2·09	14·53	40·88	4·64	29·7
	1936-37	·11	·39	·41	1·49	·060	·56	8·2	31·3	2·80	15·22	42·48	4·44	35·8
4 months............	1935-36	·097	·26	·32	1·39	·020	·45	7·0	35·5	2·03	13·66	41·81	3·82	35·8
	1936-37	·093	·25	·29	1·57	·059	·52	6·4	36·0	2·38	12·15	43·07	3·87	33·7
6 months............	1936-37	·066	·36	·36	·97	·063	·46	5·3	36·6	2·21	14·40	41·49	3·53	44·5

TABLE 11.

Average percentage composition of the dry matter of Digitaria Pentzii Pretoria Small.

Period of growth.	Season.	P.	Ca.	Mg.	K.	Na.	Cl.	Crude protein.	Crude fibre.	Ether soluble extract.	Total Ash.	N-free extract.	Silica free Ash.	Percentage dry matter of herbage as cut.
1 month.............	1935-36	·14	·51	·45	2·00	·051	·83	9·1	33·9	3·17	10·75	43·08	5·21	35·6
	1936-37	·13	·51	·44	1·57	·27	·63	8·2	33·9	3·30	10·80	43·80	4·61	32·0
2 months............	1935-36	·13	·52	·45	1·86	·040	·78	7·8	34·3	2·88	10·98	44·04	5·11	30·8
	1936-37	·11	·55	·43	1·35	·062	·59	7·3	34·8	3·27	11·86	42·77	4·15	34·2
3 months............	1935-36	·12	·46	·39	1·94	·047	·78	6·7	37·2	2·66	10·74	42·70	4·85	28·1
	1936-37	·11	·56	·42	1·24	·042	·54	6·5	35·6	3·60	11·17	43·13	4·30	37·2
4 months............	1935-36	·092	·45	·39	1·38	·022	·64	5·7	37·9	2·71	10·53	43·16	4·04	33·0
	1936-37	·098	·46	·36	1·69	·061	·63	5·8	37·3	3·26	10·40	43·24	4·29	32·4
6 months............	1936-37	·077	·54	·43	·80	·032	·40	5·0	38·5	2·93	11·20	42·37	3·72	47·7

TABLE 12.

Average percentage composition of the dry matter of Panicum maximum.

Period of growth.	Season.	P.	Ca.	Mg.	K.	Na.	Cl.	Crude protein.	Crude fibre.	Ether soluble extract.	Total Ash.	N-free extract.	Silica free Ash.	Percentage dry matter of herbage as cut.
1 month.............	1935-36	·17	·41	·46	1·41	·66	·98	10·1	30·5	2·30	13·72	43·38	5·29	29·0
	1936-37	·17	·41	·42	1·09	·52	·70	8·3	30·7	2·38	12·68	45·94	4·59	29·6
2 months............	1935-36	·12	·40	·43	1·13	·55	·87	7·0	33·7	2·26	13·62	43·42	4·68	27·6
	1936-37	·16	·42	·42	·96	·48	·59	7·2	32·1	2·35	13·45	44·90	4·15	31·3
3 months............	1935-36	·089	·35	·34	1·17	·42	·80	5·5	37·2	1·71	12·35	43·24	3·98	29·0
	1936-37	·15	·41	·41	1·03	·36	·67	6·2	33·4	2·31	12·70	45·39	4·28	33·2
4 months............	1935-36	·091	·34	·36	·87	·44	·67	4·5	38·7	1·57	12·56	42·67	3·80	31·1
	1936-37	·12	·38	·30	·82	·40	·65	6·0	34·7	2·14	10·55	46·61	3·85	33·8
6 months............	1936-37	·12	·32	·34	·93	·33	·54	4·4	39·2	1·78	11·05	43·57	3·64	41·9

(*j*) *Dry matter of herbage as cut.*—On the whole there is a tendency for the dry matter content to increase with age in the plant. With one exception, viz., the final cut for the *Digitaria species* in April, 1936, the highest percentage of dry matter was obtained in the herbage which grew undisturbed until the end of the season, i.e. the 6-monthly cuts. The rise to the highest value does not, however, proceed uniformly with decreasing frequency of cutting. Rigg and his associates (1932) referred to the profound influence of climatic conditions on the percentage of dry matter found in green pasture mown at the growth stage of 2½ inches to 3 inches in length. Drought and low temperatures retarded pasture-production and were invariably associated with high percentages of dry matter in the growth. With suitable moisture and temperature conditions, pasture production was highest, and this was accompanied by low percentages of dry matter. The influence of climate on the dry matter content of herbage in the present investigation is best illustrated by reference to tables 6 and 7, giving the seasonal production of the grasses and to tables A and E in the appendix giving the seasonal dry matter content of the herbage. A study of these tables reveals the fact that the most active growth in March, 1936, and in January and February, 1937, is associated with the lowest values for dry matter content in the course of the respective growing seasons. On the other hand, the highest percentages of dry matter are found in the herbage of the periods of drought as is evident from the figures for dry matter in the herbage cut in January, 1936, and in April, 1937. It would thus appear that while the process of drying off of mature herbage towards the end of the growing season is responsible for high values for dry matter, the amount of this constituent in the fresh herbage in the course of the growing season is largely influenced by the prevailing climatic conditions.

With reference to the seasonal composition of the grasses (see tables A and E in the appendix) the conclusion arrived at in a previous publication (1935) that " in the absence of soil and climatic deficiencies stage of growth is the main factor influencing the mineral and protein content of pasture species " appears to apply equally well to the data for this investigation. Low values for phosphorus and a relatively high level for calcium and magnesium contents are again in evidence during periods of dry weather. At the same time, the protein content does not appear to be affected adversely by unfavourable climatic conditions. In any case, the composition of monthly cut herbage cannot be considered to show marked seasonal variations. During some months a slightly more or less advanced stage of growth is attained by a species when compared with its growth of the previous month and resulting in a slight decrease or increase in the percentage content of the constituents, but, on the whole, the monthly growths of the grasses studied must be looked upon as herbage of a comparatively high composition at a comparatively early stage of growth.

In regard to the more essential constituents from the standpoint of animal nutrition the grasses studied do not show outstanding differences after the same period of growth. On the whole, for monthly cuts, *Panicum maximum* has the highest average phosphorus content, *Setaria Lindenbergiana* and *Cenchrus ciliaris* top the list for protein content, while the lowest values for these constituents are found in *Chloris gayana.* A more marked contrast in the chemical composition of these grasses lies in their potassium and sodium contents. *Chloris gayana* and *Panicum maximum* are characterised by comparatively low potassium and high sodium values, while the very opposite condition obtains in the case of the other species studied. The percentage content of potassium is up to twice as high as, and of sodium only 10 to 20 per cent. of the figures for the two species mentioned.

The average calcium-phosphorus ratio in the monthly cut herbage varies from about 2·3 in *Cenchrus ciliaris* to 3·8 in the Digitaria species, while, as may be expected from a consideration of the relative rates of decrease of the percentage of calcium and phosphorus in ageing herbage, the ratio is increased considerably with decreased frequency of cutting.

A comparison of the data for the two seasons (see tables 8 to 12) reveals some definite differences. Phosphorus, potassium, and crude protein values are lower during the second than during the first season of the experiment. On the other hand, the figures for calcium are consistently, though sometimes not markedly, higher during the 1936-37 than during the previous season. These differences may be ascribed mainly to differences in the climatic conditions obtaining during the two seasons of the experiment, and resulting chiefly in variations in the stage of growth attained by a species after the same interval of growth.

(4) THE INFLUENCE OF FREQUENCY OF CUTTING ON THE PRODUCTION OF CRUDE PROTEIN AND PHOSPHORUS.

The aggregate production of dry matter, crude protein, and phosphorus for all the species combined, but for cutting treatments separately, is given by table 13 in lb. per acre. The statistically significant features in the results relating to the effect of cutting treatment on the production of dry matter have previously been indicated. Briefly stated, they are to the effect that while there may be no difference in the yield of dry matter from certain lenient treatments the depressing influence on yield under a monthly system of cutting is significant in all cases. Due to the fact that the herbage

TABLE 13.

Total seasonal production of dry matter, crude protein, and phosphorus in lb. per acre.

	Season.	Cutting Treatment.				
		1 month.	2 months.	3 months.	4 months.	6 months.
Dry matter.......	1935–36	1,932·0	2,286·0	2,270·0	2,520·0	2,520·0
	1936–37	1,612·0	2,073·0	1,847·0	2,292·0	2,322·0
Crude protein.....	1935–36	201·0	202·0	169·0	138·0	138·0
	1936–37	136·0	149·0	116·0	123·0	102·0
Phosphorus (P)....	1935–36	2·86	2·56	2·44	1·87	1·87
	1936–37	1·79	2·16	1·82	1·98	1·63

from individual replications was not analysed separately the statistical method employed in the analysis of the yield data could not be applied to the results of the chemical analysis. The figures in table 13 giving the yield of crude protein and phosphorus will thus be taken at their face value.

The general tendency for the production of crude protein and phosphorus with different cutting treatments is just the opposite of that for the yield of dry matter, the highest yield for crude protein and phosphorus occuring in the more drastic systems of cutting. While there is no difference in protein production by the monthly and the 2-monthly systems for the 1935-36 season a steady decrease is indicated as the intervals between cuts become longer. A similar state of affairs obtains in the data for the following season except that under the monthly system of cutting the production of crude protein is actually less than under the 2-monthly system of cutting. The comparatively low production of dry matter for the three-monthly system, to which reference has been made previously, is mainly responsible for the deviation from the general tendency shown by the figures for this system in the 1936-37 season. The remarks made in regard to protein production apply equally well to the production of phosphorus, except that the highest production of phosphorus was obtained by the monthly system during the 1935-36 season.

The results for the present investigation then, confirm the conclusion reached by du Toit and his associates (1935) in a previous publication from this Institute in that, under the conditions of the experiment, the highest yield of crude protein and phosphorus is obtained when grass species are cut at intervals of two months during the growing season. Whether it may be advisable to forfeit this advantage for the sake of a higher yield of dry matter which may be obtained under more lenient systems of cutting will depend on the results of the digestibility trials to be considered later in this publication. It should be pointed out that by a monthly system of cutting the total production of crude protein and phosphorus may be of the same magnitude as that from the 2-monthly system, but the significant decrease in dry matter production and the effect of the severe treatment on the vitality of the plants remove this system from the realm of practical considerations.

To conclude this section, the markedly lower production of the nutrients under discussion for the 1936-37 season in comparison with the figures for the previous season may be briefly referred to, The tendency for lower values for phosphorus and protein in the herbage of the 1936-37 season has already been indicated. From a consideration of the data for the yield of dry matter during this season (see table 13) it appears that the decreased production of dry matter is mainly responsible for the diminished yield of crude protein and phosphorus.

In comparison with grazing by animals whose droppings are left behind to enrich the soil the utilisation of pasture as hay or silage suffers from the disadvantage that the soil is continually being depleted of nutrients whereas nothing is returned to it. It is realised that other factors may have influenced production during the 1936-37 season; the soil may not have been the most suitable for the species studied, and the climatic conditions may not have been "ideal" for maximum production. However, from purely theoretical considerations the application of fertilisers to pasture which is utilised as hay or silage becomes a necessity if continued production at a high level is aimed at. At the same time, as pointed out by Hall (1932), it may not be economically desirable to fertilise certain types of veld when the more productive and desirable grasses do not occupy 18-25 per cent. of the area.

Section B.—The Influence of Frequency of Cutting on the Digestibility and Nutritive Value of Grasses.

VII. EXPERIMENTAL DETAILS.

(*a*) The Grass Samples.

The amount of material available did not permit of feeding trials with the herbage of different cutting rotations from each grass species separately. Accordingly, the aggregate yield of herbage for the season from any one cutting treatment where the period of growth was actually that indicated by its designation, for all species combined was mixed and treated as one sample for the purpose of determining digestibility. Furthermore, since the plot experiment was continued for only four months during the 1935-36 season those portions of the plots which normally should have been harvested after six months were cut on the same date as the 4-monthly portions, so that for the said season herbage of only four different degrees of maturity was obtained. The materials may be designated as monthly, 2-monthly, 3-monthly, and 4-monthly cuts. During the 1936-37 season cutting was commenced earlier in the season with the result that herbage of five different degrees of maturity became available, namely 1, 2, 3, 4, and 6-monthly cuts. In all nine digestion trials had, therefore to be conducted.

(*b*) The Animals.

Three full-grown Merino wethers weighing on an average 70 lb. at the commencement of the investigation were employed. These animals were selected from a group of six sheep which were shorn on the 30th November, 1936, and which served from this date until the 24th December in a digestion experiment with lucerne hay. The sheep were fed at the rate of 600 grams air-dry material per day and the faeces were collected in a canvas bag, which was attached by a webbing harness, during the last nine days of the trial. The three sheep selected for the grass hay experiments continued to receive the same amount of lucerne hay until the 19th January, 1937. For ten days prior to this date the animals were placed in specially constructed metabolism crates where faeces and urine were collected daily. In this way the animals became used to the crates and, at the same time, a duplicate set of values for the digestibility of the same lucerne hay became available, namely, by collecting the faeces in a bag attached to a harness and, on the other hand, by collecting the faeces on a wire screen in a metabolism crate. The coefficients of digestibility obtained by the two methods are given below:—

	Organic matter.	Crude protein.	Ether extract.	N-free extract.	Crude fibre.
Bag collection...............	63·7	76·6	42·2	72·1	46·2
Screen collection............	65·7	76·9	43·5	73·7	49·2

In general good agreement between the results is indicated.

(*c*) The Equipment.

The metabolism crates used in the experiments were constructed locally in accordance with those employed amongst other places at the Illinois Eperimental Station for sheep and pigs. Some of the features in the construction of the crates may be briefly mentioned. The inside area on which the animal can move about is ($5\frac{3}{4}$ x 4) square feet. One end opens out into a detachable, galvanized iron feed manger. Water is supplied in a trough attached on the inside away from the feed manger. There are two false bottoms to the crate, an upper wire screen of half-inch mesh on which the animal stands and which permits the excreta to pass through. About four inches below the first floor is a second wire screen of $^1/_{16}$ inch mesh which catches the faeces and allows the urine to pass through on to the funnel-shaped real bottom, and so into a receptacle below the crate. When collections of faeces and urine have to be made the upper part and the animal are moved over on to a cleaning table hooked on the crate and supplied with screens and a funnel-shaped bottom similar to those of the crate proper. During the preliminary periods the sheep are kept in separate feeding pens measuring about (16 x 5) square feet and of which only about a third is under cover with the result that the animals may enjoy sufficient direct sunlight during all preliminary feeding periods.

(*d*) General Scheme of the Trials.

Table 14 shows the order in which the digestibility of the nine samples of herbage was tested together with the duration of the preliminary and collection periods.

TABLE 14.

Period.	Herbage sample.	Preliminary period.	Collection period.
1	4-monthly cuts, 1935–36 season........	19.1.37– 6.2.37	6.2.37–16.2.37
2	3-monthly cuts, 1935–36 season........	16.2.37–24.2.37	24.2.37– 6.3.37
3	2-monthly cuts, 1935–36 season........	6.3.37–14.3.37	14.3.37–24.3.37
4	1-monthly cuts, 1935–36 season........	24.3.37– 1.4.37	1.4.37–11.4.37
5	6-monthly cuts, 1936–37 season........	19.4.37– 2.5.37	2.5.37–12.5.37
6	4-monthly cuts, 1936–37 season........	12.5.37–20.5.37	20.5.37–30.5.37
7	3-monthly cuts, 1936–37 season........	30.5.37– 7.6.37	7.6.37–17.6.37
8	2-monthly cuts, 1936–37 season........	17.6.37–25.6.37	25.6.37– 5.7.37
9	1-monthly cuts, 1936–37 season........	5.7.37–13.7.37	13.7.37–23.7.37

In period 1 when the diet of the animals had to be changed from a protein-rich lucerne hay to a grass hay of an advanced stage of maturity the preliminary feeding interval lasted 18 days. On the 19th January the three animals, sheep 4, 5, and 6, were weighed, and from this date until the 24th of the month each animal was allowed a ration of 800 grams of air-dry hay per day, offered in two equal portions at 9 a.m. and at 3 p.m. Although none of the animals seemed to like the ration, sheep 5 and 6 consumed all the feed offered while sheep 4 refused on an average 200 grams per day, consisting of hard, fibrous portions.

The general experience on this Station has been that mature sheep, averaging 70 lb. in body weight, will not consume continuously more than about 2lb. of hay per day when they have the run of spacious camps. On the other hand, when serving in experiments and confined to small feeding pens the daily consumption was found to be in the neighbourhood of only 600 grams of hay per head. For this reason it was doubted whether the animals would continue to consume 800 grams of poor hay per day and the ration was accordingly reduced to 700 grams on the 25th January. Until the 29th sheep 4 continued to refuse from 50-100 grams of feed daily. However, from this date until the conclusion of the preliminary period on February 6 the amount left uneaten by this animal never exceeded 20 grams per day, while the other two sheep continued to consume all the feed offered. This amount, namely, 700 grams air-dry material per day, remained constant for all the digestion periods concerned in this investigation and was always offered in two equal portions in the morning and in the afternoon. Excepting periods 1, 2, 5 and 6, when sheep 4 refused small amounts of feed, clean consumption of this quantity of hay was effected.

At the conclusion of period 4 the sheep were fed for 8 days on a ration consisting of 2 parts of Teff hay and one part of lucerne hay at the rate of 700 grams per day. With the change-over from this ration, on the 19th April, to the poorest grass hay cut at the end of the 1936-37 season difficulties were again encountered with regard to consumption of the material. The sheep were obviously averse to feeding on this new ration, number 5, especially, protested violently by bleating and by kicking against the door of the feeding pen. For the next five days as much as 250 grams of hay were refused by sheep 6 per day while numbers 4 and 5 left about 100 grams per day uneaten. From the 25th April until the conclusion of the preliminary feeding period on May 2 sheep 5 and 6 consumed their rations of 700 grams daily completely, but number 4 continued to refuse about 10 grams per day. It should, however, be mentioned that the animals did not appear to relish this ration as well as that offered during the succeeding period (6). From then on until the conclusion of the trials with period 9 no difficulties were experienced and the sheep appeared to like the feed offered.

With the exception then of periods 1 and 5 when the prliminary periods lasted 18 and 14 days, respectively, the duration of these feeding intervals was 8 days and the collection of excreta was continued for 10 days for all experiments.

A preliminary period has to be of such length that the excreta can be presumed to represent the experimental ration. For this purpose a period of 8 to 10 days is generally accepted to be of sufficient duration in the case of the ruminant. Lenkeit (1932) determined the time interval taken by an experimental feed, coloured with fuchsin, to pass completely through the alimentary tract of various animals. In the case of the sheep he found that after 7 to 9 days 97 per cent. and more of the experimental feed had been passed, while complete excretion of the small amount of remaining coloured feed took 16 to 21 days with grains and 12 to 13 days with coarser fodders. Insufficient ingestion of roughage had the effect of further retarding the complete passage of an experimental ration. In view of these findings and since on the whole the difference in chemical composition between grass samples fed in successive periods in the present series of trials was small, a preliminary period of 8 days may be considered to have been of sufficient length. In any case, the amount of material available did not permit of longer preliminary periods.

(*e*) Sampling of Herbage.

Prior to commencing a digestion trial the air-dry herbage to be used in the experiment was chopped into approximately one-inch lengths with a hand forage-cutting machine. The material was then thoroughly mixed and three samples taken for chemical analysis. On two of these, weighing about 300 grams each, dry matter was determined by drying to constant weight at 100° C. in an electric oven. These samples together with the third one of about 600 grams were then finely ground in a high-speed mill, the resultant material thoroughly mixed and a representative sample taken for chemical analysis on the absolutely dry basis. At the time of taking samples for chemical analysis and dry matter determination of the hay as fed the daily rations for the whole of the preliminary and experimental periods were weighed out into paper bags. In this way fluctuations in the dry matter intake from day to day were obviated.

(*f*) Collection and sampling of the excreta.

The sheep were weighed at the beginning and at the conclusion of each experimental period. Before starting a collection period the crates were thoroughly cleaned and care was taken to remove any faecal matter adhering to the wool and hoofs of the animals before entering the crates. The exact time of day when each animal was put into its cleaned crate was noted, and taken to be the beginning of the collection period which was also concluded at the same hour.

Excreta were collected daily at 9 a.m., except for the final collection which depended on the time the animals were put into the crates at the commencement of the period.

A clean bottle was placed under the funnel of the cleaning table and the upper part of the crate and the animal moved over on to the cleaning table. After brushing down any faeces adhering to it the upper screen was removed. The lower screen with all the faeces on it was then carefully taken out and placed on clean brown paper spread on the floor of the metabolism room. Here the faeces were transferred with a scoop and brush to a large enamal tray. Bits of feed scattered by the animal were sorted out carefully and put back into the feed manger to be eaten with the next ration. Particles of crushed faeces which had passed through the lower screen were also collected and added to the main bulk of faeces. The urine receptacle under the crate was removed and replaced by a clean five-litre flask. The screens were put back into position and rinsed with about 2½ litres of distilled water using an ordinary watering can. The upper part of the crate and the animal were then pushed back on to the crate. Excreta passed by the animal while on the cleaning table were likewise collected and added to the main collections.

The urine and " washings " were mixed and 25 cc. 10 per cent. (by volume) sulphuric acid added to the mixture which was filtered through glass wool. All containers were rinsed with a little distilled water and these washings added to the main urine mixture which was made up to a definite volume, usually four litres, with distilled water. Both screens and the bottom of the crate were thoroughly scrubbed with a clean hard brush and rinsed with distilled water on the final day of collection to remove any urinary matter which might have adhered to the screens during the experimental period in spite of the daily rinsing of the screens with water. The urine and washings were usually 8 litres on the last day.

One-twentieth of the total volume of thoroughly mixed urine and washings was taken each day and stored in a glass-stoppered bottle containing a few crystals of thymol. This composite sample was analysed for nitrogen and mineral constituents at the end of the trial.

The urine container was replaced under the crate when the flask with screen washings was removed. A few thymol crystals were placed in the container as a preservative. At first acid (sulphuric or hydrochloric) was used, but as it was found to cause the precipitation of some hippuric acid its use was dispensed with.

Records were kept of water consumption as the tap water supplied to the animals contained a certain amount of lime for which a correction had, therefore, to be made in the lime intake of the animals. The sheep were fed in the morning ~~directly~~ after the completion of the collection of excreta.

The faeces from a daily collection were spread out on the enamel tray and left exposed in the metabolism room for 24 hours after which they were found to contain not more than about 12 per cent. moisture. They were then taken to the laboratory where an aliquot equal to one-tenth of the total weight of faeces was weighed out into a linen bag which was hung up in a suitable place in the laboratory. This process was repeated each day during the collection period and the daily aliquots of one-tenth of the total weight mixed in the same bag. This composite sample was weighed two or three days after the conclusion of the experiment and a sub-sample of unground faeces taken to determine its dry matter content. The main bulk of the composite sample was finely ground, thoroughly mixed, and a representative sub-sample taken for chemical analysis. Due to the fact that the moisture content of air-dry faeces may alter during the grinding process, dry matter had again to be determined on the ground material in order to have all analyses on the absolute dry basis.

Feed refused by an animal in the course of a collection period was left in the manger until the end of the trial, when it was collected, weighed, and chemically analysed. As stated previously, it was only during four of the nine digestion periods that it was found necessary to collect food residues in the case of one of the three animals.

A small quantity, about 3 grams, of table salt was scattered on the feed of each animal every morning.

VIII. RESULTS.

(*a*) THE INFLUENCE OF FREQUENCY OF CUTTING ON DIGESTIBILITY.

The chemical composition of the dry matter of the nine samples of grass hay fed during the different digestion trials is shown in table 15.

The general tendency in the variations of individual constituents with differences in the stage of growth of the herbage discussed in Section A of this paper is faithfully reflected in the composite samples, crude protein and phosphorus contents increasing with periods 1 to 4 and again with periods 5 to 9, corresponding in each case with herbage of decreasing degree of maturity.

The live-weights of individual sheep at the commencement of the trials and before and after each collection period of the series of experiments are shown in table 16.

There appears to be a slight tendency for the animals to loose weight for a short period after 12th May. This tendency is, however, not maintained and the weights registered at the conclusion of period 9 are from 5 to 7 lb. higher than those obtaining at the commencement of the trials on 19th January. There may have been a real loss in body weights due to deficiencies in the diet consumed in periods 5 and 6, but the length of time the animals were on these diets was too short to permit of definite conclusions.

TABLE 15.

Composition of dry matter of hay samples.

Period.	Hay sample.	Percentage Organic matter.	Percentage Crude protein.	Percentage Crude fibre.	Percentage Ether soluble extract.	Percentage ash.	Percentage N-free extract.	Percentage P.	Percentage Ca.	Percentage dry matter in hay as fed.
1	4-monthly cuts, 1935–36 season	89·43	5·50	38·6	1·98	10·57	43·35	0·074	0·310	91·0
2	3-monthly cuts, 1935–36 season	89·10	6·90	37·3	2·21	10·90	42·69	0·100	0·342	92·4
3	2-monthly cuts, 1935–36 season	88·60	8·84	35·0	2·35	11·40	42·41	0·112	0·393	92·4
4	1-monthly cuts, 1935–36 season	88·98	10·42	33·9	2·82	11·02	41·84	0·148	0·330	91·1
5	6-monthly cuts, 1936–37 season	87·90	4·37	36·5	2·19	12·10	44·84	0·070	0·411	91·6
6	4-monthly cuts, 1936–37 season	89·18	5·00	36·8	2·51	10·82	44·87	0·083	0·367	92·0
7	3-monthly cuts, 1936–37 season	88·13	6·28	34·0	3·00	11·87	44·85	0·099	0·441	91·7
8	2-monthly cuts, 1936–37 season	88·62	7·18	34·0	2·52	11·38	44·92	0·104	0·415	92·6
9	1-monthly cuts, 1936–37 season	88·42	8·40	34·2	2·52	11·58	43·30	0·111	0·424	90·7

TABLE 16.

Body weight record (expressed in pounds).

Period.	1			2		3		4		5		6		7		8		9	
Sheep No.	19.1.37	5.2.37	16.2.37	24.2.37	6.3.37	14.3.37	24.3.37	1.4.37	11.4.37	2.5.37	12.5.37	20.5.37	30.5.37	7.6.37	17.6.37	25.6.37	5.7.37	13.7.37	23.7.37
4	71	72	71	72	71	72	73	73	74	75	75	72	73	71	73	73	77	77	78
5	70	71	72	71	72	72	73	73	74	75	74	74	72	71	72	72	75	75	77
6	69	71	70	70	70	70	70	72	72	73	72	72	71	70	71	71	73	73	74

The amounts of the different nutrients eaten, diegsted, and voided daily by each sheep together with the co-efficients of apparent digestibility during the nine trials, when feeding the materials described in table 15 appear in table J of the appendix, table K showing the composition of the faeces. An inspection of table J reveals the fact that whilst the agreement amongst the results for the three sheep is not perfect, it may, nevertheless, be considered to be satisfactory for this type of work. On the whole sheep 4 digested its food somewhat more efficiently than did the other two animals.

In order to facilitate comparison the average coefficients of digestibility obtained for the nine samples of grass hay are summarised in table 17.

TABLE 17.

Summary of digestion coefficients (mean for 3 sheep).

Period.	Hay sample.	Organic matter.	Crude protein.	Ether extract.	N-free extract.	Crude fibre.
1	4-monthly cuts, 1935–36 season	49·2	41·3	25·6	45·0	56·3
2	3-monthly cuts, 1935–36 season	55·0	51·4	31·3	51·6	61·2
3	2-monthly cuts, 1935–36 season	58·3	60·4	30·6	55·0	64·2
4	1-monthly cuts, 1935–36 season	64·3	63·5	41·4	60·7	70·8
5	6-monthly cuts, 1936–37 season	48·7	32·4	30·9	46·5	54·2
6	4-monthly cuts, 1936–37 season	52·3	39·6	35·0	50·7	57·3
7	3-monthly cuts, 1936–37 season	58·3	50·4	39·4	57·6	62·4
8	2-monthly cuts, 1936–37 season	62·2	55·1	35·5	61·4	66·7
9	1-monthly cuts, 1936–37 season	65·8	60·3	39·4	63·6	71·9

The influence of the stage of growth on the digestibility of grasses is at once obvious from a glance at table 17. With the exception of the ether extract every constituent shows steadily improving digestibility from the herbage of the most advanced stage of maturity tested in period 1 down to the herbage at the youngest stage of growth fed in the fourth digestion period. The coefficients for the materials of the 1936-37 season vary similarly, a progressive increase taking place from digestion periods 5 to 9, corresponding with herbage of highest to lowest degree of maturity. Ether extract reveals, in general, the same tendency indicated for the other constituents but shows certain irregular fluctuations due probably to varying amounts of ether-soluble material of a metabolic character usually present in the faeces.

For the hay samples of the 1935-36 season the digestion coefficient for organic matter increases from 49·2 for 4-monthly grass through 55·0 and 58·3 for 3- and 2-monthly herbage, respectively, to the comparatively high value of 64·3 for the monthly cuts. Also, for the samples of the following season the digestibility of this constituent improves from 48·7 for the herbage cut after a growth interval of 6 months through 52·3, 58·3, and 62·2 for the intermediate systems of increasing frequency of cutting to 65·8 for the monthly cuts, the highest value obtained for the digestibility of organic matter during this series of trails.

The constituent parts of the organic matter, namely, crude protein, crude fibre, nitrogen-free extract, and, to a lesser extent, ether extract show the same variations in digestibility with stage of growth of the herbage, although differing at times in magnitude. Thus, the apparent digestibility of the crude protein appears to be influenced to a greater extent than the other constituents by the maturity of the grass. The figures for the monthly and 4-monthly herbage of the 1935-36 season differ by as much as 22·2 per cent. in favour of the herbage cut according to the former system. The improvement in digestibility with increasing frequency of cutting is as pronounced in the samples for the following season, the lowest value being 32·4 for the 6-monthly cuts as against 60·3 for the monthly cuts. The composition of the crude fibre content of the herbage samples may have influenced the apparent digestibility of the crude protein in that, although the crude fibre content as determined for two different grass samples may be of the same magnitude, there may be differences in the amounts of indigestible, encrusting materials, such as lignin, which would protect portions of the protein in some measure from enzymatic activity. Nevertheless, from a comparison of the relevant figures in tables 15 and 17 there appears to be a closer relationship between the crude protein content of a sample and its apparent digestibility with constant dry matter intake. In any case, the apparent digestibility of the crude protein of the herbage samples tested remains an index of maturity.

Comparing again table 15 giving the percentage composition of the dry matter of the grass samples tested with table 17 it would appear that there is no consistent relation between the percentage of crude fibre in the hay consumed and the coefficient of digestibility. For digestion periods 1 to 4 the digestibility of the crude fibre improves progressively with decreasing percentage of crude fibre in herbage obtained under increasing frequency of cutting. But, for digestion periods 5 to 9, whilst the digestibility increases in the same progressive manner with the shortening of intervals between cuts, the crude fibre content of the herbage remains the same for periods 5 and 6, then drops somewhat but is again constant for the three final periods. It appears, therefore, that for the climatic conditions obtaining during the two seasons of the plot experiment the figure for crude fibre content as determined by the conventional method does not as such indicate the extent to which it will be digested. More knowledge is needed in regard to the actual chemical composition of the crude fibre moiety. The results of work recently undertaken by Norman (1936) show that " the cellulosic structure of a plant is underestimated in an irregular manner in the fibre determination ". Whereas in young herbage the crude figure accounted for about 90 per cent. of the cellulose, in more mature herbage only 72 per cent. was estimated. A contributary factor in this was found to be the increase of xylan in the cellulose with increasing age. Lignin was also found to increase with maturity.

Whilst, therefore, as stated when discussing the digestibility of the crude protein, the actual figure for crude fibre may be of a similar magnitude in two different herbage samples, the changes taking place in the structural materials of plants with age will result in decreased digestibility of the crude fibre.

The digestion coefficients for the nitrogen free extract show the same tendency with the stage of growth in the samples as indicated for the other constituent parts of the organic matter. However, the nitrogen-free extract is distinctly less digestible than the crude fibre, the coefficients being on an average 10·0 per cent. and 6·6 per cent. higher for the latter than for the former constituent in the hay samples of the 1935-36 and the 1936-37 seasons, respectively.

Woodman (1930) found a similar result for a mineral-deficient herbage tested by him. He remarked that " although this finding emphasizes the important rôle played by bacteria in the digestion of roughages by ruminants, it is difficult to understand why the nitrogen-free extractives in such herbage should run off more quickly than the fibrous constituents into an indigestible and unavailable condition ". The result is perhaps not so surprising when considered in conjunction with the possibility that fermentation in the animal may change crude fibre so as to be partly soluble in 1·25 per cent. acid or alkali, thereby causing it to appear in the nitrogen-free extract of the faeces, according to the conventional method of calculating the so-called " nitrogen-free extractives ". Low figures for the crude fibre content of the faeces will consequently be partly responsible for high values for computed nitrogen-free extract content of the faeces (see table K, appendix), and this in turn will cause the coefficients of digestibility for nitrogen-free extract to be low.

Fraps (1930) studied the digestibility by sheep of the constituents of the nitrogen-free extract of feeds. He found, for instance, that the nitrogen-free extract of roughages contained high percentages of pentosans and other residues not included under sugars, starches and pentosans determined; and that these pentosans and residues of the nitrogen-free extract were digested considerably less efficiently than those occurring in the N-free extract of concentrates. In any case, it seems evident that the nature of the constituents of the N-free extract and the crude fibre of roughages offers a field for extensive investigation.

The coefficients of digestibility for the ether extract, the remaining constituent in table 17, do not show consistent variations with degree of maturity of the herbage, and are comparatively low. These low values and a certain degree of lack of uniformity may be ascribed to the low percentage of ether extract in the hays studied and to the fact that the faeces always contain ether-soluble material of a metabolic character, as previously stated.

Due to the intimate relationship existing between the stage of growth of the herbage and its digestibility comparisons between the results of this investigation and those obtained by other workers are complicated. However, in table 18 a few such comparisons are made with the results obtained in period 4 of the present series of trials.

TABLE 18.

Showing the coefficients of digestibility of roughages.

Roughage.	Percentage Crude Protein.	Percentage Crude Fibre.	Percentage digested.				
			Organic Matter.	Crude Protein.	Ether Extract.	N-free Extract.	Crude Fibre.
Teff hay (Ross, et al, 1927)………………….	9·8	31·9	—	61·2	22·9	57·8	67·1
Rhodes grass hay (Iyer, 1935)………………….	11·4	37·5	63·7	59·4	24·8	55·3	74·4
Prairie hay (Christensen, 1932)………………	9·6	28·3	64·3	52·2	33·6	67·5	66·8
Mineral-deficient hay (Woodman, 1930)……….	13·0	35·1	48·0	52·0	5·8	41·8	57·1
Period 4 (This investigation)……………….	10·4	33·9	64·3	63·5	41·4	60·7	70·8

TABLE 19.

Showing percentages digestible nutrients and starch equivalent in dry matter of hay samples fed during digestion periods 1 to 9.

Period.	Hay sample.	Dig. Crude Protein.	Dig. Organic Matter.	Dig. Ether Extract.	Dig. Crude Fibre.	Dig. N-free Extract.	P.S.E.	Nutritive ratio.
1	4-monthly cuts, 1935–36 season……………………	2·27	44·00	0·51	21·72	19·50	21·92	1 : 18·6
2	3-monthly cuts, 1935–36 season……………………	3·55	49·00	0·69	22·80	22·02	27·88	1 : 13·0
3	2-monthly cuts, 1935–36 season……………………	5·34	51·65	0·72	22·47	23·32	31·89	1 : 8·8
4	1-monthly cuts, 1935–36 season……………………	6·61	57·22	1·17	24·00	25·40	38·18	1 : 7·8
5	6-monthly cuts, 1936–37 season……………………	1·42	42·80	0·68	19·78	20·87	22·11	1 : 29·6
6	4-monthly cuts, 1936–37 season……………………	1·98	46·52	0·88	21·10	22·75	26·04	1 : 23·0
7	3-monthly cuts, 1936–37 season……………………	3·17	51·40	1·18	21·20	25·83	32·54	1 : 15·6
8	2-monthly cuts, 1936–37 season……………………	3·96	55·15	0·89	22·68	27·60	35·98	1 : 13·1
9	1-monthly cuts, 1936–37 season……………………	5·07	58·20	0·99	24·60	27·52	38·92	1 : 10·6

A South African, an Indian, a European, and an American roughage have been selected, and the crude protein and crude fibre contents have been included in the table to indicate the approximate stage of growth.

A distinct feature in the table is, with the exception of the prairie hay, the higher coefficients of digestibility for crude fibre in relation to those for the N-free extract. The ether extract values are comparatively low in all cases, crude protein has been digested somewhat more efficiently in the South African roughages, and the digestibility of the organic matter is distinctly low in the European mineral deficient hay in comparison with that for other hays reported in the table. In any case, the digestibility of the South African fodders compares favourably with that of materials of a somewhat similar stage of growth in other countries.

(*b*) THE INFLUENCE OF FREQUENCY OF CUTTING ON NUTRITIVE VALUE.

Table 19 gives a summary of the percentages of digestible nutrients and starch equivalent in the dry matter of the herbage samples tested in digestion periods 1 to 9. The starch equivalent has been calculated by the usual formula, namely (digestible protein × 0·94) plus (digestible fat × 1·91) plus (digestible crude fibre) plus (digestible nitrogen-free extract). Since "true" protein has not been determined the figure for digestible crude protein has been employed. To the value obtained according to the above formula the customary correction for roughages (Kellner, 1915) of a deduction of 0·58 of a starch equivalent for each 1 per cent. of total crude fibre in the hay sample concerned has been applied to arrive at the figures for production starch equivalent (P.S.E.) in table 19. From the method of computation it is evident that the figures represent net energy values, one pound of starch equivalent, according to Kellner, having a net energy value of 1,071 calories.

The results in table 19 again bring out the influence of the stage of growth of the herbage on its feeding value. The dry matter of the 4-monthly cuts fed in period 1 contains only 44·00 per cent. digestible organic matter including 2·27 per cent. digestible crude protein as against 57·22 per cent. digestible organic matter including 6·61 per cent. digestible crude protein in the herbage of period 4, cut at monthly intervals during the 1935-36 season. In the hay samples of the following season the digestible organic matter increases in a similarly progressive manner from 42·80 per cent., including only 1·42 per cent. digestible crude protein in the 6-monthly cuts to 58·20 per cent. of which 5·07 per cent. is digestible crude protein, in the herbage from the highest frequency of cutting fed in the final period of the series of trials. The nutritive ratio is extremely wide in most cases, but is profoundly influenced by the stage of growth of the grass samples. For the 1935-36 samples it decreases from 18·6 to the medium value of 7·8 in the monthly cuts, while for the 1936-37 samples it remains wide for all systems of cutting, but, nevertheless decreases from 29·6 in the oldest to 10·6 in the youngest herbage.

As may be expected, considering the method of computation, the starch equivalents vary similarly to the digestible organic matter in the hay samples studied, the values increasing from 21·92 to 38·18 and from 22·11 to 38·92 with increasing frequency of cutting in the herbage samples of the 1935-36 and 1936-37 seasons, respectively.

Following a procedure employed by Woodman (1930) the amounts of starch equivalent which the sheep were consuming during the periods of seemingly increasing liveweight were calculated. For this purpose digestion periods 3 and 4 and digestion periods 8 and 9 were chosen(cf table 16). These two sets of periods were each of about four weeks duration, namely, from the 14th March to the 11th April and from the 25th June to the 23rd July, to be henceforth known as period A and period B, respectively. The average daily consumption of dry matter per head was 1·41 lb. for period A and the same amount, viz. 1·41 lb., for period B (cf. table J). The mean starch equivalent content of the herbage fed during the two periods was 35·03 and 37·45 for periods A and B, respectively, so that the mean daily starch equivalent consumption of the sheep amounted to 0·49 lb. in period A and 0·53 lb. in period B, and on this they were able fully to maintain live weight. The mean weights of the sheep (average for three animals) were 72·3 lb. in period A and 74·4 lb. in period B. Employing Rubner's formula, viz. $E_1 = E_0\left(\frac{V_1}{V_0}\right)^{\frac{2}{3}}$, where E_1 and E_0 relate to energy consumption and V_1 and V_0 to the corresponding live-weights, the figures above for starch equivalent consumption become 0·61 lb. and 0·65 lb. for a sheep of 100 lb. live-weight in periods A and B respectively.

Wood and Capstick (1926) gave a figure of 1·26 lb. of starch equivalent as the maintenance allowance for a sheep weighing 100 lb., while Woodman (1930) indicated that 0·86 lb. of starch equivalent enabled a sheep to fully maintain 100 lb. live-weight. The mean for the figures computed from the data of the present investigation, namely, 0·63 lb. for a sheep weighing 100 lb. is exactly 50 per cent. of Wood and Capstick's figure and considerably lower than the value calculated by Woodman. On the other hand, Møllgaard (1931) in his book "Grundzüge der Ernährungsphysiologie der Haustiere" gave a figure of 708 NK_F as the maintenance requirement of a sheep weighing 50 Kg. This, converted by the expressions 2365 NK_F = 1 Kg. starch equivalent and $E_1 = E_0\left(\frac{V_1}{V_0}\right)^{\frac{2}{3}}$ becomes 0·62 lb. of starch equivalent per 100 lb. live-weight, a figure which is practically the same as that obtained above. However, Møllgaard's figure has been taken from the older German investigations and the figure of 0·63 above has been computed from the data of a short term trial under experimental conditions, and may for that reason be an underestimation of the net energy requirements for maintenance, if the other figures quoted in the above discussion are to be considered as standard values. In this connection it should be pointed out that the figure here obtained has been calculated from the data from the herbage samples of the lowest degree of maturity, and it will consequently be still lower if computed from the data obtained in periods 1, 2, 5, 6, and 7. If, therefore, 0·63 lb. starch equivalent is an under-estimation of the maintenance requirement, the animals must have been on a sub-maintenance ration for the whole of the series of trials, and such a conclusion is unacceptable in view of the fact that the animals may be considered to have at least maintained body-weights for the period January 19 to July 23, a period of fully 6 months during which they subsisted entirely on the experimental rations, except for the 8 days between periods 4 and 5 when they were fed on the Teff-lucerne hay mixture, the composition of which did not differ materially from that of the grass hay consumed during period 4.

Nevertheless, whilst the results of the present investigation seem to indicate that the animals employed are able to maintain live-weight on much smaller amounts of starch equivalent than are demanded by European standards, they do ,on the other hand, focus attention on the necessity for long-term trials with the object of establishing whether samples of grass hay obtained according to the systems of cutting applied during the two seasons of the experiment will at the same level of dry matter intake provide sufficient net energy to maintain live-weight, irrespective of the stage of growth of the grasses, and disregarding for the moment other deficiencies which may be present in herbage of an advanced stage of maturity, such as protein and phosphorus. The present series of trials aimed primarily at determining digestibility and were not planned for the purpose of establishing the relative energy value of herbage of varying degree of maturity for the maintenance of sheep, but they do indicate the necessity for utilising grasses at the younger stages of growth.

To conclude this discussion it will be interesting to compile a new table from table 13, giving the production of dry matter and crude protein under the different frequencies of cutting applied to the grasses, in the light of the data relating to digestion coefficients in table 17. Table 20 shows the production of digestible crude protein and starch equivalent from each of the series of cutting treatments to which the grasses were subjected.

In view of the influence of cutting frequency on the digestibility and gross production of pasture species discussed earlier it may be expected to affect the yield of digestible nutrients in a similar manner, only to a more marked degree. A glance at table 20 reveals the fact that while there may be no difference between the monthly and the 2-monthly systems, the production of digestible nutrients from any one of these treatments is superior to that from the more lenient systems of cutting. The effect of repeated defoliations at monthly intervals on the vitality and yielding capacity of the grasses is not reflected in the yield of digestible nutrients for the first season of the experiment but becomes distinctly noticable during the second season, when there was a difference of about 20 per cent. in the production of starch equivalent in favour of the 2-monthly system of cutting. In view of this circumstance there again seems to be justifiable grounds for the conclusion reached earlier in this report, namely that, under the climatic conditions of the experiment, a system of cutting natural veld grasses at intervals of 2 months during the growing season will result in the optimum utilisation of such pasture.

TABLE 20.

Seasonal production of digestible crude protein and starch equivalent in pounds per acre.

	Season.	Cutting treatment.				
		1 month.	2 months.	3 months.	4 months.	6 months.
Digestible crude protein.......	1935–36 1936–37	128 82	122 82	87 58	57 49	57 33
Starch equivalent.............	1935–36 1936–37	738 627	729 746	632 601	552 596	552 514

(c) THE INFLUENCE OF FREQUENCY OF CUTTING ON THE NITROGEN, PHOSPHORUS AND CALCIUM BALANCES.

The nitrogen, phosphorus, and calcium balances for each of the series of nine digestion trials appear in tables L, M and N of the appendix, the period numbers corresponding with those in Table 14.

Considering the average of the mean daily balances for the three sheep it is seen that, except for period 5 when a negative balance was obtained, the amount of hay consumed provided sufficient nitrogen to maintain the sheep in nitrogen equilibrium and to permit of the storage of nitrogen in the animal organism during some of the periods of the series.

From the data given in Table 21 it is possible to arrive at an approximate figure for the nitrogen maintenance requirement of a fullgrown Merino wether weighing about 70 lb. (cf. Table 16). From an inspection of Table 21 it may be concluded that during digestion periods 1 and 6 the sheep were on an average in nitrogen equilibrium, the amounts of nitrogen digested being 2·29 grams and 2·03 grams respectively. The mean value for the two periods, viz. 2·16 grams digestible nitrogen is assumed to be adequate for the maintenance of nitrogen equilibrium. Since, however, the wool of the sheep always grows allowance for the nitrogen thus stored should be made in the maintenance requirement.

Møllgaard (1931) gives an average amount of 14·0 grams digestible protein to be the additional requirement for wool growth of a sheep weighing 100 kg., or proportionally, 0·72 grams digestible nitrogen for a sheep of 70 lb. live weight. The total requirement for maintenance and wool growth would then be 2·88 grams digestible nitrogen or 18·0 grams digestible crude protein per day for a sheep weighing 70 lb. According to Wood and Woodman's "Rations for Live Stock" (1932) a sheep of similar weight would require 0·28 lb. per week or 18·2 grams digestible protein per day. It should, however, be mentioned that the figures in their table for protein are given in terms of "protein equivalent".

TABLE 21.

Digestion Period.	Hay sample fed.	Percentage Crude Protein in dry matter.	Actual mean daily dry matter intake (gms.).	Mean daily N. intake (gms.).	Coefficient of apparent digestibility.	Mean daily N. digested (gms.).	Mean daily N. balance (gms.).	Calculated daily dry matter intake required for N. maintenance (gms.).
1	4-monthly cuts, 1935–36 season	5·50	631	5·54	41·3	2·29	+ 0·16	794
2	3-monthly cuts, 1935–36 season	6·90	656	7·12	51·4	3·66	+ 0·77	516
3	2-monthly cuts, 1935–36 season	8·84	647	9·15	60·4	5·52	+ 2·41	338
4	1-monthly cuts, 1935–36 season	10·42	638	10·63	63·5	6·75	+ 1·80	272
5	6-monthly cuts, 1936–37 season	4·37	638	4·45	32·4	1·44	− 0·30	1,276
6	4-monthly cuts, 1936–37 season	5·00	641	5·12	39·6	2·03	+ 0·13	908
7	3-monthly cuts, 1936–37 season	6·28	642	6·45	50·4	3·25	+ 1·10	569
8	2-monthly cuts, 1936–37 season	7·18	648	7·45	55·1	4·11	+ 1·52	454
9	1-monthly cuts, 1936–37 season	8·40	635	8·53	60·3	5·14	+ 1·51	356

TABLE 22.

Digestion Period.	Mean body weight of sheep (Kg.).	Mean daily dry matter intake (gms.).	Mean daily nitrogen intake (gms.).	Mean daily total faecal nitrogen (gms.).	Mean daily metabolic faecal nitrogen (gms.).	Coefficient of apparent digestibility.	Coefficient of true digestibility.	Mean daily nitrogen absorbed (gms.).	Mean daily urinary nitrogen (gms.).	Mean daily endogenous urinary nitrogen (gms.).	Mean daily nitrogen utilised (gms.).	Percentage utilization.
1	32·3	631	5·54	3·25	1·77	41·3	73·2	4·06	2·13	1·03	2·96	72·9
2	32·3	656	7·12	3·48	1·84	51·4	78·4	5·58	2·89	1·03	3·72	66·7
3	32·7	647	9·15	3·63	1·81	60·4	80·2	7·33	3·12	1·04	5·25	71·6
4	33·2	638	10·63	3·88	1·79	63·5	80·4	8·54	4·94	1·06	4·66	54·6
5	33·6	638	4·45	3·01	1·79	32·4	72·6	3·23	1·75	1·08	2·56	79·3
6	32·7	641	5·12	3·09	1·79	39·6	74·6	3·82	1·91	1·05	2·96	77·5
7	32·3	642	6·45	3·19	1·80	50·4	78·5	5·06	2·16	1·04	3·94	77·8
8	33·2	648	7·45	3·35	1·81	55·1	79·3	5·91	2·59	1·07	4·39	74·3
9	34·5	635	8·53	3·39	1·78	60·3	81·2	6·92	3·63	1·10	4·39	63·4

The figure calculated above would be lower if given in similar terms, depending on the pure protein content of the rations of grass hay consumed, but would nevertheless be in close agreement with that given in "Rations for Live Stock". The amount of material available did not permit of duplication of individual trials to establish whether the nitrogen balances observed would persist indefinitely. At the same time, there seems to be ground for the point of view that in the case of protein the growth obtained in a few weeks of feeding or the rate of nitrogen retention in even shorter periods under properly controlled conditions, will be representative of the adequacy of the protein supply [Mitchell and Hamilton (1929)].

In any case, even if it is only approximate, the figure calculated may serve the purpose of determining the relative values of the hay samples fed during digestion periods 1-9, as sources of nitrogen for the maintenance requirements of the Merino sheep. These values appear in the last column of Table 21 and represent the weight of dry matter which a 70 lb. sheep must consume daily of any one of the different hay samples to ensure the ingestion of 18·0 grams digestible crude protein, i.e. the requirement for maintenance and wool growth. The figures afford yet another demonstration of the profound influence of frequency of cutting on the nutritive value of grasses. For instance, in the case of the samples of the 1936-37 season, a sheep has to consume no less than about 1300 grams of the dry matter of the 6-monthly cuts for its nitrogen requirements, whereas the nitrogen in only 356 grams of the dry matter of monthly cuts is adequate for the same needs.

However, the dry matter intake remained practically the same for all the periods in the series of experiments and the plane of nitrogen intake consequently varied from period to period. Under these conditions it will be interesting if the percentage utilization or biological value of the nitrogen at the different planes of intake corresponding with herbage obtained under different frequencies of cutting were known.

In the nitrogen balance method described by Mitchell (1923) the biological value of a protein is defined as the percentage of the absorbed nitrogen which is retained by the body for repair or the construction of nitogenous tissue. Due to the fact that a certain fraction of the faecal nitrogen is derived from the intestinal tract and its secretions during digestion the ordinary coefficient of digestibility discussed earlier in this paper is not a true measure of the amount of nitrogen absorbed; the undigested food nitrogen only, and not the total nitrogen in the faeces, should be deducted from the nitrogen intake to obtain the amount actually absorbed. Furthermore, the urinary excretions contain nitrogen resulting from tissue catabolism as well as nitrogen resulting from incomplete utilization of dietary nitrogen. It is obvious, therefore, that the body's contribution of nitrogen to the urinary and faecal excretions must be assessed to permit of the measurement of the percentage utilization of the nitrogen absorbed.

Various direct methods have been devised for separating the two forms of faecal nitrogen by digestion of the faeces with enzymes or special solvents, [Pfeiffer and Lemmermann (1901); Wöhlbier

(1930)] but these are considered to be based on unacceptable assumptions and do not give the information desired [Woodman (1924). Mitchell and Hamilton (1929), Watson and Horton (1936)]. Several workers in the field of nutrition are now making use of indirect methods for the estimation of the endogenous urinary faecal nitrogen. [Mitchell (1923), Sotola (1930), Martin and Robinson (1922), Boas Fixsen (1930), Hutchinson and Morris (1936)]. Briefly the principle of this method resolves itself into the measurement of the faecal and urinary excretion of nitrogen when the animal consumes a nitrogen-free ration complete in all respects except for the absence of nitrogen. The body's contribution of nitrogen to the urinary and faecal excretions during periods of protein feeding is then measured by the nitrogen content of the urine and faeces of the experimental animal when receiving a nitrogen free ration, the feed intake being such as to provide liberally for the energy maintenance requirement. It has also been shown that the excretion of nitrogen in the faeces of animals on nitrogen-free diets varies directly with the amount of feed consumed.

The present series of trials were not planned for the purpose of measuring biological values, but, since both the endogenous urinary nitrogen and the metabolic faecal nitrogen of the same three sheep (employed in these trials) were determined by Smuts (1938) in experiments following directly on the digestion trials, the values obtained by him are assumed to be a fair indication of the endogenous nitrogen metabolism of the animals during the digestion experiments reported in this paper. Smuts fed a nitrogen-free ration containing about 25 per cent. crude fibre and found the average endogenous urinary and faecal nitrogen of the three animals to be 0·032 grams nitrogen per kilogram body weight and 0·28 grams per 100 grams food intake, respectively. Using these figures the percentage utilization of the nitrogen estimated to have been absorbed from the rations fed in digestion periods 1 to 9 has been calculated in Table 22 from the experimental data shown in Table 16 and Tables J and L of the appendix. Average values for the three sheep have been used throughout.

A glance at Table 22 shows that the figures for the "true" digestibility greatly exceeds those for the "apparent" digestibility, the differences between the two series of values becoming less as the nitrogen content of the feed consumed increases. The accuracy of the figures for the digestibility is, of course, dependent upon the accuracy of the figure for the metabolic faecal nitrogen, and since the determination of the latter is based upon an indirect method of uncertain exactness, the values for the true digestibility must be looked upon as only approximate. At the same time a slight under or overestimation of the metabolic faecal nitrogen will not seriously affect the value calculated for the percentage of nitrogen utilised by the animal body, as both the numerator and the denominator of the fraction determining the biological value are influenced by the figure for the metabolic faecal nitrogen.

The accepted influence of the level of nitrogen intake on its utilisation by the animal organism [Mitchell (1923)] is clearly demonstrated by the variations in the figures for the percentage utilisation in Table 22 with variations in the nitrogen concentration of the rations. With increasing intake in periods 1 to 4, and again in periods 5 to 9 the percentage utilisation tends to decrease, the highest and lowest values being indicated for periods 5 and 4 when the nitrogen intake was at its lowest and highest, respectively.

Whilst, therefore,. the actual amount of nitrogen utilised for maintenance and tissue growth was highest during those periods when grasses at the younger stages of growth were fed, (see column 12, Table 22), the wastage of nitrogen in the metabolic processes of the animal was least when the rations consisted of more mature herbage. If the level of nitrogen intake had been the same for each of the nine hay samples of varying degree of maturity tested, the percentage utilisation of the nitrogen absorbed may be expected to have been of a somewhat similar magnitude throughout. On the other hand, from what has been stated earlier in this paper under "general scheme of the trials" with reference to the amount of grass hay which the type of sheep employed in the experiments will consume continuously, it is doubtful whether the animal will consume the weight of 6 monthly cut grass hay which is needed for its nitrogen maintenance requirements (see Table 21). At the same time the relatively small amount of hay obtained by cutting at monthly intervals, which will suffice for its nitrogen needs, will probably not be adequate to provide for its energy requirements. Consequently, if grass hay has to be made use of, without the addition of other energy-yielding or nitrogenous supplements, it is concluded that it may be utilised most advantageously, as a maintenance ration for adult sheep, when cut at the intermediate stages of growth, viz. at 2- or 3- monthly intervals.

Owing to the shortness of the feeding experiments and since the inadequacy of nutrients that may be stored in considerable amounts relative to current expenditure in the animal body may be deferred for longer periods of time than in the case of protein, there is little justification for embarking upon a discussion of the results obtained in the measurements of the calcium and phosphorus balances. These balances have been included with the other results of the investigation merely to indicate probable influences of the different rations on the mineral metabolism of the animals within the short interval of time allotted to each digestion trial.

The data for the calcium balances have been decidedly disappointing in that consistently negative balances were obtained, in spite of variations in the mineral content of the rations consumed. In considering these results it should be borne in mind that mature sheep were employed in the experiments. The maintenance requirements for such animals does not appear to be definitely known [Franklin (1935)], but is undoubtedly small relative to that for growing animals. Factors which may have influenced the calcium balances are the digestibility or availability of the calcium in, the unfavourable calcium-phosphorus ratio of, actual deficiency of phosphorus during some periods, and the probable absence of unknown factors in the experimental rations.

Apparently the grass samples obtained by cutting at monthly and 2-monthly intervals and fed during periods 4 and 9, and periods 3 and 8, respectively, tended to bring about a condition of phosphorus equilibrium in the animals, whereas negative balances were registered for all other periods. In view of the advantages attaching to a system of cutting herbage at 2-monthly intervals during the growing season, as indicated previously, the mineral metabolism of animals subsisting entirely on such herbage could with advantage be investigated with special reference to the requirements for maintenance and growth in respect of calcium and phosphorus.

IX. GENERAL SUMMARY AND CONCLUSIONS.

Five species of indigenous grasses, namely *Chloris gayana*, *Setaria Lindenbergiana*, *Cenchrus Ciliaris*, *Digitaria Pentzii Pretoria small*, and *Panicum maximum*, were established each in five replications on 25 plots, each measuring (24 × 17) square feet, in a Latin Square arrangement. Each plot was again subdivided into five equal portions and a different treatment allocated at random to each subplot within a main plot. The effective cutting area of a subplot measured (22 × 3) square feet. The experiment covered two growing seasons and the treatments applied were:—

(1) Cutting the herbage at monthly intervals;
(2) Cutting the herbage at 2-monthly intervals;
(3) Cutting the herbage at 3-monthly intervals;
(4) Cutting the herbage after 4 months growth, followed by an aftermath cut of 2 months growth; and
(5) Cutting the herbage once only towards the end of the growing season, usually a 6-monthly cut.

The objects of the investigation were to study the effect of the treatments on the yield, chemical composition, digestibility and nutritive value of the grasses under natural conditions of soil and climate. To test the digestibility the produce obtained under any one cutting treatment for all the species combined was treated as a single sample. Three adult Merino wethers were employed and the hay samples were tested in nine successive digestion periods.

The following is a summary of the results obtained:—

(1) Grasses of the stoloniferous type were readily propagated from roots, whereas the establishment of bunch grasses by this method demanded much care and attention.
(2) The intrusion of weeds and other grass species indigenous to the locality on the established pure stands occurred most on those sub-plots cut at monthly intervals.
(3) Of the grass species studied *Setaria Lindenbergiana* and *Panicum maximum* gave the highest yields of dry matter.
(4) The yield of dry matter was lowest when cutting the grasses at monthly intervals during the growing season.

(5) Of the more lenient cutting treatments the 6- and 4-monthly systems produced on the whole more dry matter than the 2- and 3-monthly systems.

(6) Evidence which suggests that the amount of dry matter obtainable under a 2-monthly system of cutting may not differ materially from that resulting from one cut at the end of the season or two cuts as represented by a 4-monthly cut in February followed by an aftermath cut two months afterwards, has been obtained.

(7) Rainfall is the most important factor governing the yield of grass under natural conditions. It would appear that there is a period of optimum growth which is independent of the rainfall during seasons when the latter is normal in amount and distribution.

(8) The content of phosphorus, potassium, chlorine, and crude protein of the dry matter of herbage samples is markedly influenced by cutting treatments, the values decreasing with decreasing frequency of cutting. Calcium and magnesium values do not show appreciable variation with stage of growth, while the general tendency for sodium is to decrease with age in the plant. The crude fibre content varies more or less inversely with the protein content and the percentage ether-soluble extract shows irregular fluctuations with variations in the stage of growth. The nitrogen-free extract shows little variation in the dry matter of herbage from different cutting treatments.

(9) On the whole the dry matter content of grasses increases with age in the plant. It is, however, considerably influenced by climatic conditions. Drought is associated with high values while suitable moisture and temperature conditions causing active growth are favourable to low values for dry matter content.

(10) While there may be no difference in the production of crude protein and phosphorus under a monthly and a 2-monthly system of cutting, a steady decrease in the production of these nutrients is indicated as the intervals between cuts become longer.

(11) Except for the ether-soluble extract which occasionally shows irregular fluctuations, the coefficients of apparent digestibility for all the constituents of the organic matter of the hay samples decrease steadily with advancing stage of growth. The crude fibre was found to be more digestible than the nitrogen-free extract.

(12) Lengthening the interval between cuts greatly influenced the nutritive value. The dry matter of 6-monthly cut herbage contained only 22·11 per cent. starch equivalent including 1·42 per cent. digestible crude protein as against 38·92 per cent. starch equivalent including 5·07 per cent. digestible crude protein in the dry matter of monthly cuts.

(13) The nutritive ratio remained wide, but improved from 1:29·6 to 1:10·6 in the dry matter of 6-monthly and monthly cut herbage, respectively.

(14) The data of the digestion trials seem to suggest that the animals employed were able to maintain live-weight on much smaller amounts of starch equivalent than are demanded by European standards. The figure calculated is 0·63 lb. of starch equivalent per day for a sheep weighing 100 lb., and confined to a metabolism crate.

(15) The production of digestible nutrients obtainable by cutting grasses at monthly and 2-monthly intervals is considerably higher than that resulting from the more lenient systems of cutting. The unfavourable effect of repeated defoliations at monthly intervals on the vitality of the plants is reflected in a higher yield of starch equivalent under the 2-monthly system during the second season of the experiment.

(16) With practically the same dry matter intake for all the samples of hay tested the concentration of nitrogen in the rations consumed varied according to the stage of growth from digestion period to period. Under these conditions the most economic utilisation of the nitrogen absorbed occurred during those periods when the animals were consuming herbage of the highest degree of maturity containing the lowest percentage of nitrogen. However, a sheep will probably not consume sufficient of such poor and comparatively unpalatable hay to provide for its nitrogen maintenance requiremnts.

(17) The consistently negative calcium balances obtained were probably not due to an absolute deficiency of this mineral in the grass samples. No explanation is given for the phenomenon.

(18) The animals appeared to have been in a state of phosphorus equilibrium when feeding on herbage cut at monthly and 2-monthly intervals.

Since the results obtained with the grass species studied on the particular type of soil and under the climatic conditions obtaining during the two seasons of the investigation may not be taken to apply generally, it is not possible to lay down clear directions for the practical man. Nevertheless, from a consideration of the evidence of the data obtained in this experiment as a whole it seems to be a warrantable conclusion that a system of cutting grasses at approximately 2-monthly intervals during the growing season for the purpose of providing feed during times of scarcity will result in the most economical utilisation of indigenous grass species under natural conditions.

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	ty ter ut.	Description of Grasses as Cut.
Chloris gayana	·1	Short, pre-flowering, wilted and scorched in parts by sun.
	7·2	Short, a few flowerheads appearing.
	·7	Short, flowering.
	7·8	Short, a few flowerheads appearing.
Setaria lindenb	·2	Some with flowerheads, wilted and scorched in parts.
	7·6	Some with flowerheads.
	5·1	Pre-flowering.
	1·3	Pre-flowering.
Cenchrus ciliar	·4	Short, some with flowerheads.
	4·0	Short, pre-flowering.
	4·3	Short, few flowerheads appearing.
	3·7	Short, preflowering.
Digitaria pentzi	·8	Short, pre-flowering, wilted.
	0·0	Short, pre-flowering.
	0·6	Short, few flowerheads appearing.
	·1	Short, pre-flowering.
Panicum, max	0·2	Short, few flowerheads present, wilted.
	3·2	Flowerheads appearing.
	1·4	Flowering.
	3·2	Short, few flowerheads appearing.

	ry atter Cut.	Description of Grasses as Cut.
Chloris gayana	1·8	Flowering.
	9·9	Long, with flowerheads.
Setaria lindenb	5·0	Long, flowering.
	1·4	Long, no flowerheads.
Cenchrus ciliar	8·4	Flowering.
	1·5	Few flowerheads present.
Digitaria pentzi	2·5	Short, few flowerheads present.
	9·2	Few flowerheads present.
Panicum, max	6·5	Flowering.
	8·8	Long, with flowerheads.

ested and voided.

Sheep 5.				Sheep 6.				
Crude Protein (gm.).	Ether Extract (gm.).	N-free Extract (gm.).	Crude Fibre (gm.).	Dry Matter (gm.).	Organic Matter (gm.).	Crude Protein (gm.).	Ether Extract (gm.).	N-free Extract (gm.).
35·04	12·62	276·5	246·0	637·0	569·5	35·04	12·62	276·5
19·65	9·34	153·0	110·7	352·2	292·2	21·54	9·48	152·8
15·39	3·28	123·5	135·3	284·8	277·3	13·50	3·14	123·7
43·9	26·0	44·6	55·0	44·7	48·7	38·5	24·9	44·7
44·63	14·30	276·3	241·5	646·8	576·3	44·63	14·30	276·3
21·37	9·73	136·1	96·0	314·0	259·2	22·28	9·73	133·3
23·26	4·57	140·2	145·5	332·8	317·1	22·35	4·57	143·0
52·1	31·9	50·8	60·2	51·4	55·0	50·0	31·9	51·8
57·20	15·20	274·3	226·4	646·8	573·0	57·20	15·20	274·3
22·11	10·58	124·1	78·2	301·5	244·2	22·98	10·74	125·1
35·09	4·62	150·2	148·2	345·3	328·8	34·22	4·46	149·2
61·3	30·4	54·8	65·4	53·4	57·4	59·9	29·4	54·4
66·45	17·96	266·6	216·2	637·7	567·6	66·45	17·96	266·6
24·20	10·30	106·2	62·2	257·7	206·2	24·62	10·68	106·2
42·25	7·66	160·4	154·0	380·0	361·4	41·83	7·28	160·4
63·6	42·7	60·2	71·2	59·6	63·7	62·9	40·5	60·2
27·98	14·04	287·5	234·0	641·2	563·6	27·98	14·04	287·5
18·58	9·42	155·4	110·2	370·6	299·1	19·45	9·86	159·6
9·40	4·62	132·1	123·8	270·6	264·5	8·53	4·18	127·9
33·6	32·9	46·0	52·9	42·2	47·0	30·5	29·8	44·5
32·16	16·16	289·0	237·0	644·0	574·3	32·16	16·16	289·0
19·38	10·48	148·8	104·2	330·9	271·9	19·83	10·52	139·0
12·78	5·68	140·2	132·8	313·1	302·4	12·33	5·64	150·0
39·7	35·1	48·5	56·0	48·6	52·6	38·3	34·9	51·9
40·28	19·26	288·0	218·2	641·9	565·7	40·28	19·26	288·0
19·96	11·30	121·8	83·6	301·7	236·6	20·01	11·85	122·8
20·32	7·96	166·2	134·6	340·2	329·1	20·27	7·41	165·2
50·4	41·3	57·7	61·6	53·0	58·2	50·2	38·5	57·4
46·59	16·33	291·2	220·4	648·2	574·5	46·59	16·33	291·2
20·67	10·17	112·6	72·0	283·5	222·6	21·48	10·69	113·8
25·92	6·16	178·6	148·4	364·7	351·9	25·11	5·64	177·4
55·6	37·7	61·3	67·2	56·2	61·2	53·9	34·5	60·8
53·33	16·00	275·0	217·1	634·9	561·4	53·33	16·00	275·0
20·50	9·67	99·3	56·4	263·5	202·1	21·88	9·96	102·5
32·83	6·33	175·7	160·7	371·4	359·3	31·45	6·04	172·5
61·6	39·6	63·9	73·9	58·5	64·0	59·0	37·7	62·7

TABLE K.

Showing composition of dry matter of faeces collected during digestion periods 1-9.

Period.	Sheep No.	Percentage Crude Protein.	Percentage Crude Fibre.	Percentage Ether Extract.	Percentage Ash.	Percentage N-free Extract.	Percentage P.	Percentage Ca.
1...........	4	6·0	30·3	2·73	16·98	44·00	0·172	0·742
	5	5·6	31·4	2·65	16·95	43·40	0·173	0·754
	6	6·1	30·7	2·69	17·10	43·40	0·186	0·760
2...........	4	6·9	29·4	3·22	17·87	42·5	0·228	0·834
	5	6·7	30·1	3·05	17·48	42·7	0·211	0·822
	6	7·1	29·8	3·10	17·45	42·5	0·225	0·809
3...........	4	7·9	27·3	3·57	19·32	41·9	0·237	0·976
	5	7·5	26·7	3·61	19·75	42·4	0·235	0·968
	6	7·6	28·3	3·56	19·00	41·5	0·235	0·936
4...........	4	9·6	25·0	4·24	20·30	40·9	0·281	0·972
	5	9·5	24·4	4·04	20·40	41·7	0·282	1·015
	6	9·6	25·1	4·15	19·96	41·2	0·283	0·976
5...........	4	5·42	29·2	2·84	20·15	42·39	0·160	0·835
	5	5·11	30·3	2·59	19·30	42·70	0·159	0·826
	6	5·25	29·7	2·66	19·30	43·09	0·155	0·818
6...........	4	5·88	30·0	3·25	17·76	43·11	0·188	0·805
	5	5·65	30·4	3·06	17·50	43·39	0·191	0·812
	6	6·00	31·0	3·18	17·83	42·00	0·193	0·818
7...........	4	6·68	26·9	3·98	21·50	40·94	0·217	1·023
	5	6·64	27·8	3·75	21·30	40·51	0·220	1·018
	6	6·64	27·1	3·93	21·60	40·73	0·219	1·005
8...........	4	7·56	26·0	3·93	22·12	40·39	0·237	1·060
	5	7·48	26·1	3·68	21·95	40·79	0·235	1·046
	6	7·58	27·0	3·77	21·50	40·15	0·235	1·017
9...........	4	8·60	23·6	3·84	23·95	40·00	0·265	1·115
	5	8·36	23·0	3·94	24·26	40·44	0·267	1·130
	6	8·30	25·7	3·78	23·32	38·90	0·263	1·075

TABLE L.

Showing Nitrogen balances in sheep during digestion periods 1-9.

Period.	Sheep No.	N. consumed per day (gm.).	N. excreted per day.			Mean daily N-balances (gm.).
			Faeces (gm.).	Urine (gm.).	Total (gm.).	
1...............	4	5·42	3·17	2·06	5·23	+ 0·19
	5	5·61	3·14	2·06	5·20	+ 0·41
	6	5·61	3·44	2·28	5·72	− 0·11
2...............	4	7·09	3·41	2·83	6·24	+ 0·85
	5	7·14	3·42	3·02	6·44	+ 0·70
	6	7·14	3·56	2·83	6·39	+ 0·75
3...............	4	9·15	3·67	2·95	6·62	+ 2·53
	5	9·15	3·54	3·20	6·74	+ 2·41
	6	9·15	3·67	3·20	6·87	+ 2·28
4...............	4	10·63	3·84	4·84	8·68	+ 1·95
	5	10·63	3·87	4·94	8·81	+ 1·82
	6	10·63	3·94	5·05	8·99	+ 1·64
5...............	4	4·41	2·95	1·78	4·73	− 0·32
	5	4·48	2·97	1·78	4·75	− 0·27
	6	4·48	3·11	1·69	4·80	− 0·32
6...............	4	5·07	3·00	2·02	5·02	+ 0·05
	5	5·15	3·10	1·85	4·95	+ 0·20
	6	5·15	3·17	1·85	5·02	+ 0·13
7...............	4	6·45	3·18	2·22	5·40	+ 1·05
	5	6·45	3·19	2·16	5·35	+ 1·10
	6	6·45	3·21	2·09	5·30	+ 1·15
8...............	4	7·45	3·30	2·58	5·88	+ 1·57
	5	7·45	3·30	2·59	5·89	+ 1·56
	6	7·45	3·43	2·59	6·02	+ 1·43
9...............	4	8·53	3·39	3·57	6·96	+ 1·57
	5	8·53	3·28	3·70	6·98	+ 1·55
	6	8·53	3·50	3·63	7·13	+ 1·40

TABLE M.

Showing Phosphorus balances in sheep during digestion periods 1-9.

Period.	Sheep No.	P. consumed per day (gm.).	P. excreted per day.			Mean daily P-balance (gm.).
			Faeces (gm.).	Urine (gm.).	Total (gm.).	
1	4	0·46	0·57	0·005	0·58	− 0·12
	5	0·47	0·61	0·004	0·61	− 0·14
	6	0·47	0·65	0·004	0·65	− 0·18
2	4	0·64	0·71	0·004	0·71	− 0·07
	5	0·65	0·68	0·004	0·68	− 0·03
	6	0·65	0·71	0·004	0·71	− 0·06
3	4	0·72	0·69	0·005	0·70	+ 0·02
	5	0·72	0·69	0·004	0·69	+ 0·03
	6	0·72	0·71	0·004	0·71	+ 0·01
4	4	0·94	0·70	0·006	0·71	+ 0·23
	5	0·94	0·72	0·004	0·72	+ 0·22
	6	0·94	0·73	0·004	0·73	+ 0·21
5	4	0·44	0·55	0·004	0·55	− 0·11
	5	0·45	0·58	0·004	0·58	− 0·13
	6	0·45	0·58	0·003	0·58	− 0·13
6	4	0·53	0·60	0·004	0·60	− 0·07
	5	0·54	0·65	0·004	0·65	− 0·11
	6	0·54	0·64	0·003	0·64	− 0·10
7	4	0·64	0·64	0·005	0·65	− 0·01
	5	0·64	0·66	0·004	0·66	− 0·02
	6	0·64	0·66	0·004	0·66	− 0·02
8	4	0·67	0·65	0·005	0·66	+ 0·01
	5	0·67	0·65	0·005	0·66	+ 0·01
	6	0·67	0·67	0·004	0·67	0·00
9	4	0·70	0·65	0·006	0·66	+ 0·04
	5	0·70	0·65	0·005	0·66	+ 0·04
	6	0·70	0·69	0·004	0·69	+ 0·01

TABLE N.

Showing Calcium balances in sheep during digestion periods 1-9.

Period.	Sheep No.	Ca. consumed per day.			Ca. excreted per day.			Mean daily Ca. balance (gm.).
		Food (gm.).	Water (gm.).	Total (gm.).	Faeces (gm.).	Urine (gm.).	Total (gm.).	
1.........	4	1·90	0·08	1·98	2·46	0·15	2·61	− 0·63
	5	1·97	0·08	2·05	2·66	0·13	2·79	− 0·74
	6	1·97	0·07	2·04	2·67	0·20	2·87	− 0·83
2.........	4	2·20	0·09	2·29	2·58	0·16	2·74	− 0·45
	5	2·21	0·08	2·29	2·62	0·10	2·72	− 0·43
	6	2·21	0·08	2·29	2·54	0·16	2·70	− 0·41
3.........	4	2·54	0·09	2·63	2·83	0·21	3·04	− 0·41
	5	2·54	0·09	2·63	2·83	0·17	3·00	− 0·37
	6	2·54	0·08	2·62	2·82	0·20	3·02	− 0·40
4.........	4	2·10	0·09	2·19	2·43	0·14	2·57	− 0·38
	5	2·10	0·08	2·18	2·58	0·13	2·71	− 0·53
	6	2·10	0·08	2·18	2·51	0·17	2·68	− 0·50
5.........	4	2·59	0·07	2·66	2·83	0·33	3·16	− 0·50
	5	2·63	0·07	2·70	3·01	0·24	3·25	− 0·55
	6	2·63	0·06	2·69	3·03	0·29	3·32	− 0·63
6.........	4	2·33	0·08	2·41	2·56	0·21	2·77	− 0·36
	5	2·36	0·07	2·43	2·78	0·15	2·93	− 0·50
	6	2·36	0·06	2·42	2·71	0·22	2·93	− 0·51
7.........	4	2·83	0·08	2·91	3·04	0·33	3·37	− 0·46
	5	2·83	0·07	2·90	3·06	0·24	3·30	− 0·40
	6	2·83	0·07	2·90	3·03	0·32	3·35	− 0·45
8.........	4	2·69	0·08	2·77	2·89	0·35	3·24	− 0·47
	5	2·69	0·07	2·76	2·89	0·23	3·12	− 0·36
	6	2·69	0·07	2·76	2·88	0·31	3·19	− 0·43
9.........	4	2·69	0·07	2·76	2·75	0·25	3·00	− 0·24
	5	2·69	0·07	2·76	2·77	0·19	2·96	− 0·20
	6	2·69	0·06	2·75	2·83	0·24	3·07	− 0·32

Section VI.

Toxicology.

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The Toxicity of some Dipping Fluids containing Arsenic and Sulphur.

By DOUW G. STEYN, Section of Pharmacology and Toxicology, and P. M. BEKKER, Section of Chemical Pathology, Onderstepoort.

I. ARSENICAL DIPPING FLUIDS.

ARSENICAL dipping fluids are very extensively used in the Union of South Africa in the eradication of ticks, lice and scab mites. The occurrence of so many cases of accidental arsenical poisoning in stock is therefore not surprising. In view of all the cases of accidental poisoning with dipping fluids the information supplied in this paper will be of interest.

A. SODIUM ARSENITE.

The following solutions of sodium arsenite are employed on a very large scale as dipping fluids for cattle and sheep:—

(*a*) Three-day dipping fluid containing 0·08 gm. of As_2O_3 per 100 c.c. Of this fluid approximately 1,200 to 2,500 c.c. (= ±2 to ±4 pints) would be fatal for a full-grown beast and 300-600 c.c. (= ±½ to ±1 pint) for a sheep.

(*b*) Seven-day dipping fluid containing 0·16 gm. of As_2O_3 per 100 c.c. Of this fluid a fatal quantity for a full-grown beast would be approximately 600 to 1,250 c.c. (= ±1 to 2 pints) and for a sheep 150 to 300 c.c. (= ±¼ to ½ pint).

(*c*) Fourteen-day dipping fluid containing 0·24 gm. of As_2O_3 per 100 c.c. Of this fluid approximately 400 to 800 c.c. (=⅔ to 1⅓ pint) would be fatal for a full-grown beast and 100 to 200 c.c. (= ±⅙ to ⅓ pint) for a sheep.

B. COOPER'S POWDER.

Cooper and Nephews (South Africa), Limited, are the manufacturers of (*a*) Cooper's Double Dipping Powder and (*b*) Cooper's Powder.

(a) *Cooper's Double Dipping Powder.*

The following is the registered composition of Cooper's Double Dipping Powder:—

Total arsenic equivalent to	9·4 per cent. As_2O_3.
Free sulphur	57·5 per cent.
Combined sulphur	6·1 per cent.
Total sulphur	63·6 per cent.

TABLE I.

Experiments with Cooper's Double Dipping Powder.

Rabbit No.	Weight in Kg.	Quantity of Cooper's Double Dipping Powder given.	Result.
A.	3·0	0·2 gm. of dry powder per os.	Transient apathy and loss of appetite which lasted for about 2 days.
B.	1·9	0·5 gm. of dry powder per os.	Listlessness and loss of appetite set in within four hours after dosing; on the following day there were pronounced apathy, profuse diarrhoea, marked paresis, especially of hindquarters, laboured respiration, and weak and accelerated pulse. Death occurred within 36 hours after dosing. *P.M. appearances :* General cyanosis ; hyperaemia of lungs ; pronounced acute catarrhal gastroenteritis ; fatty degeneration of the liver and kidneys.
C.	2·8	0·5 gm. of dry powder per os.	Symptoms and post mortem appearances similar to those described in B. Death occurred seven days after dosing.
D.	2·25	1·0 gm. of dry powder per os.	Symptoms of poisoning as described above set in within three hours after dosing. Death occurred 8 hours after dosing.
E.	2·1	1·5 gm. of dry powder per os.	Profuse diarrhoea within 5 hrs. after dosing ; the animal died 6 hours after dosing. *P.M. appearances :* pronounced acute haemorrhagic gastroenteritis, pronounced hyperaemia of lungs and liver ; further as in B.
F.	2·22	2·5 gm. of dry powder per os.	Profuse diarrhoea within 4 hrs., death occurring within 6 hrs. *P.M. appearances :* as in E.
G.	2·22	15 c.c. (= 0·2 gm.) of dipping strength solution per stomach tube.	Result as in A.

TABLE I (*continued*).

Rabbit No.	Weight in Kg.	Quantity of Cooper's Dipping Powder given.	Result.
H.	2·15	37·5 c.c. (= 0·5 gm.) of dipping-strength solution per stomach tube.	Result as in B.
I.	2·05	37·5 c.c. (= 0·5 gm.) of dipping-strength solution per stomach tube.	Symptoms and post mortem appearances as in B. Death occurred within 70 hours after dosing.
J.	2·05	75·0 c.c. (= 1·0 gm.) of dipping-strength solution per stomach tube.	Died 4½ hours after dosing. Symptoms and post mortem appearances as in E.
K.	2·36	75·0 c.c. (= 1·0 gm.) of dipping-strength solution per stomach tube.	Died 6 hours after dosing. Symptoms and post mortem appearances as in F.
L.	2·82	112·0 c.c. (= 1·5 gm.) of dipping-strength solution per stomach tube.	Profuse diarrhoea within 3 hours after dosing and death within 4 hours. Symptoms and post mortem appearances as in E with the gastroenteritis of a less acute nature.

From the above table it could be concluded that 0·15 gm. of Cooper's Double Dipping Powder per Kg. of body weight constitutes the *m.l.d.* for the rabbit. As the powder is stated to contain 9·4 per cent. of As_2O_3 the *m.l.d.* of the arsenic (expressed as As_2O_3) present in it is approximately 0·014 gm. per Kg. of body-weight for rabbits.

(*b*) *Cooper's Dip.*

The registered composition of Cooper's Dip is as follows:—"Arsenic present as trioxide = 12·5 per cent. As_2O_3, arsenic present as pentoxide = 6·0 per cent. As_2O_3, arsenic present as sulphides and sulphur-arsenic complexes = 4·0 per cent. As_2O_3. Sodium combined with arsenic = 4·9 per cent. Na_2O. Total sulphur = 68·0 per cent.".

The Cooper's Powder was administered per stomach tube in a small quantity of water.

TABLE II.

Experiment with Cooper's Powder.

Rabbit No.	Weight in Kg.	Quantity of Cooper's Dip administered.	Result.
A.	1·15	0·075 gm.	The animal developed transient loss of appetite and diarrhoea lasting approximately four days.
B.	1·75	0·075 gm.	Same symptoms* as in A but to a less marked degree.
C.	1·7	0·15 gm.	Died within 2½ hours after dosing.
D.	1·65	0·15 gm.	Died within 36 hours after dosing.
E.	2·05	0·3 gm.	Died within 27 hours after dosing.
F.	1·9	0·3 gm.	Died within 28 hours after dosing.
G.	1·6	0·5 gm.	Died within 5 hours after dosing.
H.	1·8	0·5 gm.	Died within 15 hours after dosing.

* The symptoms are similar to those described in Table I.

From the above table it is evident that 0·06 gm. of Cooper's Powder (= ±0·014 gm. As_2O_3) could be considered the approximate *m.l.d.* per Kg. body-weight of rabbit.

From the results of the above experiments it appears that Cooper's Powder given *per os* is approximately two and a half times as toxic as Cooper's Double Dipping Powder. This naturally does not apply to cases where animals are dipped in solutions prepared with these two powders.

According to experiments conducted by Theiler (1912) the *m.l.d.* of Cooper's Dip for sheep appears to be approximately 2·0 gm.

From the results of a large number of experiments conducted upon rabbits by one of us (D. G. S.) with different arsenical preparations (sodium arsenite, Cooper's Dip, Cooper's Double Dipping Powder, Rademeyer's Locust Poison) it appears that they all possess approximately the same degree of toxicity when the toxic doses are calculated on the bases of their As_2O_3 content, namely 0·014 gm. As_2O_3 per Kg. body-weight for rabbits.

Sheep, cattle and horses are more susceptible than rabbits to the effects of arsenic.

It is obviously impossible to lay down a definite figure as *m.l.d.* of an arsenical preparation for any particular class of animal as its degree of toxicity depends upon:—

(1) Its degree of solubility in water;

(2) whether water is drunk soon after having ingested it, or not;

(3) whether taken on a full stomach, or not; and

(4) whether vomiting occurs after ingestion, or not.

II. SULPHUR DIPS.

CAPEX.

This is a liquid lime-sulphur dip, consisting of polysulphides of calcium [generally $CaSx(X = 4 \cdot 6)$], manufactured by Messrs. Cape Explosives, Somerset West, C.P. The specimen of undiluted dipping-fluid employed in our experiments contained 34·47 per cent. of polysulphide sulphur and the diluted dip (dipping-strength) contained 1·5 per cent. of polysulphide sulphur.

Capex is very extensively used in the eradication of scab in sheep and mange in goats.

The following table (III) is a summary of the drenching experiments conducted with Capex upon rabbits and sheep.

TABLE III.

Experiments with undiluted and diluted (dipping-srtength) Capex.*

Animal No.	Weight in Kg.	Quantity of Capex administered per stomach tube.	Result.
Rabbit A.	1·95	2·5 c.c. of undiluted Capex on 16.6.37.	Laboured respiration (costo-abdominal), loss of appetite, and an accelerated and strong pulse persisted for 2 days after drenching.
Rabbit B.	2·6	5·0 c.c. of undiluted Capex on 16.6.37.	The following symptoms of poisoning set in within 7 minutes after drenching :—pronounced laboured respiration (deep, costo-abdominal and accelerated), pronounced restlessness, pronounced general cyanosis, general convulsions, muscular fibrilations. Death occurred 7 minutes after drenching, the heart stopping about 2 mins. after cessation of respiration. After the heart had stopped the animal still showed a few general clonic spasms (asphyxia ?). *P.M. appearances :* Pronounced general cyanosis ; pronounced hyperaemia of the lungs and liver, which was almost black in colour, blood dirty-brown in colour, large amount of sulphur precipitated in the stomach and small intestine. No evidence of gastro-intestinal irritation.
Rabbit C.	2·25	10 c.c. of undiluted Capex on 16.6.37.	The animal died within 3 minutes after drenching with symptoms and post mortem appearances as described in rabbit B.
Rabbit D.	2·1	30 c.c. of undiluted Capex on 16.6.37.	Death occurred within 2 minutes with symptoms and post mortem appearances as described in rabbit B.
Rabbit E.	1·95	60 c.c. of undiluted Capex on 16.6.37.	Result identical with that described in rabbit D.

TABLE III (*continued*).

Animal No.	Weight in Kg.	Quantity of Capex administered per stomach tube.	Result.
Rabbit F.	2·55	10 c.c. of diluted Capex.	Apart from transient loss of appetite the animal developed no symptoms of ill-health.
Rabbit G.	2·2	30 c.c. of diluted Capex.	Symptoms of pronounced dyspnoea and accelerated heart-beat set in within a few minutes and death occurred within ½-hour after dosing with symptoms and post mortem appearances as in B.
Rabbit H.	2·35	60 c.c. of diluted Capex.	Death occurred within 4 hours after drenching with symptoms and post mortem appearances as described in rabbit B.
Rabbit I.	2·5	120 c.c. of diluted Capex.	Result similar to that in rabbit H, death occurring within 8 minutes.
Sheep 49206.	6-tooth 50 Kg.	1,000 c.c. of diluted Capex.	Symptoms of pronounced dyspnoea set in within 4 minutes ; a few minutes later the animal staggered about as if intoxicated ; pronounced acceleration of heart action ; about 6 hours after drenching the animal showed considerable improvement, there being only slight laboured respiration and apathy ; when chased it soon became exhausted. In spite of this improvement the animal died during the night about 18 hours after drenching. *P.M. appearances :* Pronounced odour of sulphuretted hydrogen from the nostrils ; pronounced general cyanosis ; blood of a fairly dirty-brown colour ; subepicardial, subendocardial and intramyocardial haemorrhages ; pronounced hyperaemia and slight oedema of the lungs ; extensive haemorrhage into the subperitoneal, peritracheal and perioesophageal tissues and into the intercostal muscles ; pronounced fatty degeneration of the liver ; gastrointestinal contents dark in colour and contains a large amount of precipitated sulphur ; haemorrhages in duodenal mucosa.
Sheep 37511.	Full-mouth. 51 Kg.	3,000 c.c. of diluted Capex.	Very pronounced dyspnoea within 2 minutes ; the animal staggered and fell down and was unconscious within twenty minutes after drenching ; there were cyanosis and tremors of the superficial muscles of the body ; respiration was accelerated and superficial ; the heart beat was weak and slow (70 p.m.), eventually it became very irregular and stopped 25 minutes after drenching. The respiration ceased about 2 minutes before the heart-beat. *P.M. appearances :* Pronounced general cyanosis ; blood of an intense dirty-brown colour with delayed coagulation time ; numerous subepicardial haemorrhages ; hyperamia of the liver and lungs ; dilation of both heart ventricles ; large quantity of sulphur precipitated in the rumen, reticulum and small intestine with pronounced odour of H_2S ; hyperaemia of abomasal mucosa of duodenum, jejunum and anterior portion of the ileum, with petechiae ; hyperaemia of caecal mucosa.

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* Containing 1·5 per cent. of polysulphide sulphur.

It thus appears that the *m.l.d.* of the undiluted dipping-fluid for the rabbit is approximately 1·5 c.c. per Kg. of body-weight. Of the diluted fluid (dipping-strength) the *m.l.d.* for rabbits is approximately 7·5 c.c. per Kg. of body-weight and approximately 800 to 1,000 c.c. for a full-grown sheep weighing approximately 40 to 50 Kg.

If drawn into the lungs (choking) sulphur dipping-fluids are extremely poisonous.

According to comparative tests conducted upon rabbits it appeared that the following sulphur dipping-fluids (dipping-strength concentrations) possess the same degree of toxicity as the Capex Lime Sulphur Dip:—

(1) Tarzan Lime Sulphur Dip;* manufactured by Cape Chemical Company, Limited, Capetown. Thiosulphate (+tetrathionate) = 0·17 per cent. $[CaSx(X = 4{\cdot}39)]$. It contains 35·46 per cent. of polysulphide sulphur; and

(2) Nelson's Sodium Polysulphide Dip* $[Na_2Sx(X = 4{\cdot}27)]$. It contains 46·91 per cent. of polysulphide sulphur. In all three these dipping-fluids (dipping-strength concentration) the percentage of polysulphide sulphur is 1·5.

TABLE IV.

Sulphur Determinations made upon the Blood of Sheep 37511 *and* 49206.

Sheep No.	Date of Bleeding.	Total sulphur in mg. per 100 c.c. blood.
37511	25.6.37	85·15 83·77 } done in duplicate
	29.6.37—(*a*) before dosing................	67·30
	(*b*) 10–15 mins. after dosing 3 litres of Capex Lime Sulphur Dip.†	75·54
	(*c*) Heart-blood collected ± 1½ hours after death..	98·88
49206	25·6·37................................	95·45
	29.6.37—(*a*) before dosing................	63·87
	(*b*) 20 mins. after dosing 1 litre of Capex Lime Sulphur Dip†.	65·24
	(*c*) Death occurred during the following night and heart blood was collected the following morning.	93·40

† Analysis of Capex Lime Sulphur Dip used in dosing the sheep:—(1) 1·5 per cent. of polysulphide sulphur; (2) 0·24 per cent. of thiosulphate (including tetrathionate sulphur).

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* All the figures quoted here are those obtained with the specimens employed in our experiments.

The two sheep were bled on the 25.6.37 and again a few hours before dosing on 29.6.37 in order to determine the normal value for total sulphur in the blood. From the above table it is evident that the values obtained on the 25.6.37 were much higher than those of the 29.6.37 prior to dosing. The values for both sheep, however, showed an agreement on the same day, namely 85·15 and 95·45 on the 25.6.37 and 67·30 and 63·87 mg. sulphur per 100 c.c. blood on the 29.6.37 respectively. All the determinations were made in the same manner using the method of Stockholm and Koch (*Jour. Amer. Chem. Soc.*, Vol. 45, 1923, p. 1953). Equal quantities of the same reagents were used in every case and in addition duplicate blank determinations, which differed only in the fourth decimal place, were made.

It will be noticed that in the case of both sheep there was a pronounced increase in the sulphur values of the specimens of blood collected a few minutes before dosing and those collected after dosing. There was, however, not such a marked difference in these values of specimens of blood collected a few days before dosing and those collected after death. It is also evident that the blood sulphur values increased at a more rapid rate in the sheep which had received the larger dose of Capex. It is fully realised that the number of sheep in the experiment and the number of determinations made upon the blood sulphur are very limited and that it would be futile to attempt to draw any definite conclusions.

We did expect to find a fairly pronounced sulphaemoglobinaemia. Spectroscopic examination of the blood of the sheep however did not reveal it. This does not necessarily mean that no sulphaemoglobin was present as it may have been present in indetectable quantities. Experiments *in vitro* yielded interesting results. Normal blood, which showed the haemoglobin bands spectroscopically, showed the broad band of reduced haemoglobin after addition of a small quantity of Capex Lime Sulphur Dip. Upon shaking this mixture up with air the oxy-haemoglobin bands reappeared and after standing for a while the oxy-haemoglobin was again reduced by the sulphur dip. If a small quantity of Capex dip was added to normal blood and left standing for a while both the sulphaemoglobin band and the broad band of reduced haemoglobin could be clearly seen.

SUMMARY.

1. Experiments to determine the toxic doses of some dipping-fluids containing arsenic and sulphur are described.

2. It appears that in all the more common forms of arsenical preparations, which are soluble in water, the *m.l.d.* for rabbits can be calculated on the basis of approximately 0·014 gm. of As_2O_3 per Kg. of body-weight. Sheep, cattle and horses are more susceptible than rabbits to arsenic.

3. The *m.l.d.* of diluted (dipping-strength concentration) Capex Lime Sulphur Dip for rabbits is approximately 7·5 c.c. per Kg. of body-weight and for sheep approximately 15 to 20 c.c. per Kg. of body-weight.

4. In dipping-strength concentration Tarzan Lime Sulphur Dip and Nelson's Sodium Polysulphide Dip possess approximately the same degree of toxicity as Capex Lime Sulphur Dip.

REFERENCE.

THEILER, A. (1912). Experiments to determine the safe dose of white arsenic, Cooper's Dip and bluestone for sheep. *South Afr. Agric. Jour.*, Vol. 3, 1912, pp. 321-351.

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* Now out of print.

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Section I.

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Immunization against Rinderpest, with Special Reference to the use of Dried Goat Spleen.

By G. PFAFF, Veterinary Research Laboratory, Insein, Burma.

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I. INTRODUCTION.

IN little more than two years rinderpest reduced the cattle population of the Cape of Good Hope by 35 per cent. In the eighteenth century the disease killed over thirty million cattle in Germany alone, and not until towards the close of the nineteenth century was it eradicated from the greater part of Europe, where for hundreds of years it had been the most serious plague of animals. In many parts of the world segregation of the healthy and slaughter of the sick, which had proved successful in Europe, is not practicable, and in Burma and India, and presumably also in many other tropical countries, rinderpest remains the most important disease of cattle.

During the last forty-five years immunization against rinderpest has claimed the attention of numerous workers and has been the subject of many publications. The object of this paper is to describe a dried vaccine which is cheap, safe and effective, and which in the past two years has been successfully used on over three quarters of a million cattle.

II. IMMUNIZATION AGAINST RINDERPEST.

(*a*) *Early attempts with Virus.*

From about 1754, attempts to protect cattle were made by inserting under their skin, material soaked in the nasal and ocular discharges of diseased animals. With fairly resistant cattle this method attained a certain measure of success, but in most parts of Europe it caused heavy losses. In 1872 the International Veterinary Conference considered that rinderpest should be dealt with by segregation and slaughter, and that immunization should not be attempted, thus indicating that little progress had been made in control methods since A.D. 69 when Columella, in describing a disease which appeared to be rinderpest, recommended the segregation of sick animals.

(*b*) *The use of Bile.*

The spread of the disease to Southern Africa in the nineties of the last century attracted many workers, and from that time dates the intensive study of the problem of immunization against rinderpest.

In the South African outbreak Koch (1897) and others [Edington (1899) Kolle and Turner (1898), Kohlstock (1897), and Theiler (1897)] confirmed the claims made by cattle owners for the protective value of the bile of infected animals. The value of bile was influenced by the stage at which it was collected, the blood content, and the temperature and delays to which it was exposed before use. The effectiveness of bile was limited to two days and Theiler considered the immunity it conferred did not last more than three months. Bile contained both antibodies and virus and by adding glycerine Edington (1899) probably killed the virus, thus converting the bile method into passive immunization lasting only about ten days. Kohlstock (1897) recommended virulent blood about a fortnight after the bile. Bile was extensively used during the South African epidemic but towards the end was replaced by the serum alone and the serum-virus methods. Nevertheless, in December 1903 the first Pan-African Veterinary Conference resolved that failing an adequate supply of serum, pure bile should be used for stamping out outbreaks of rinderpest (Curson 1936).

(*c*) *The use of Serum.*

The protective value of serum from recovered animals had been noted by Semmer (1893). This was later confirmed by Koch (1897), Theiler, and others, and from that time until recently the production of immune and hyperimmune serum has been studied by many, and numerous publications have appeared on the subject Hyperimmunization and other means of obtaining potent serum were adopted in nearly all countries. Some workers, however, maintained that the serum of recovered animals was as good as that supplied by hyperimmunized animals. Edwards (1925 and 1927c) affirmed that for the production of potent serum hyperimmunization was unnecessary and in India this process was finally abandoned in 1924. He showed that susceptible buffaloes given virus and simultaneously sufficient serum to suppress reaction developed a solid immunity but their serum had negligible protective properties. If the quantity of serum was sufficiently reduced to allow the animal to develop a mild but decided reaction, including a pronounced temperature reaction, the antibody content of the blood rose sharply on the 18th day, was sustained until the 36th day, and then gradually dropped until the 60th to the 90th day. No matter how severe the clinical reaction, the antibody content of the blood was not increased. Following the work of Madsen (1923) in Copenhagen, Edwards injected intravenously the chlorides of manganese, zinc, magnesium, barium, and copper, and pilocarpine and found that " the salting 2 hours before bleeding of the serum producers when they are still yielding a good serum seems to cause them to furnish a somewhat better serum; later when they are yielding a poor serum the salting does not increase materially the quality of the serum."

Serum protects for little more than 10 days and the cost of conferring this transient immunity should restrict its use to areas where the disease is not endemic and where cattle movements are under control, or for immunizing cattle passing through an infected area. Bederke (1931) reports it little used because of bad results and Delpy (1935a) rightly says that it is too expensive and the immunity conferred too transient. Nevertheless in Syria serum alone is said to be superior to serum-virus and to vaccine (Said 1934). There is still a considerable demand for serum, either alone or in combination with virus or vaccine. In the Punjab, for instance, in 1936-37 it was given to 58,917 animals either alone or with goat virus (Quirke 1937); and in Kenya in 1934 to 99,245 animals either alone or with virus (Brassey-Edwards 1936). The number of doses of serum issued from Muktesar during the year ending 31st March, 1937, was 1,227,628 (Taylor 1938).

(*d*) *The Serum-virus Method.*

The earliest forms of the serum-virus method appear to be those of Edington (1899) who gave glycerinated bile followed by blood virus, and Kohlstock (1897) who recommended virulent blood 2 weeks after bile. Before the end of the South African epidemic this was replaced by the serum and virulent blood method, and in 1898 Kolle and Turner recommended serum-virus simultaneously as the best method for the control of the disease. Much of the work after this related to increasing the antibody content of the serum, and has been briefly referred to in connection with the serum alone method.

The disadvantages of the serum-virus method are:—

1. the limited viability of the virus,
2. the spread of the disease by starting new centres of infection,
3. the losses due to protozoa injected with the virus, and
4. the high cost of serum.

Many workers have remarked on the limited viability of the virus. Hall (1933) considered that in blood the virus remained effective for only four hours, and Walker (1921) showed that although the virus after withdrawal from the animal could give rise to a reaction it did not necessarily confer immunity. In 1929 he recommended that virulent blood should not be used later than four hours after withdrawal from the virus producer. To overcome this disadvantage several procedures were tried. One was to obtain the virus from infected animals in the herd to be immunized. Another was to infect susceptible cattle or goats and despatch them to the scene of inoculation by train or lorry. Blood was sent out on ice, but even then it was not always satisfactory when used within 24 hours (Walker 1929). Edwards (1927a), seeking to overcome the disadvantage of limited viability by the application of the work of Kligler and Robertson (1922) on the spirochaete of relapsing fever, found that in blood diluted with an equal quantity of diluted horse serum (1:2) and kept in tubes sealed with paraffin wax the virus retained its potency for 10 days at 37° C. He, therefore, despatched the virus in bottles nearly filled with defibrinated blood and sealed with plugs of cotton wool soaked in paraffin wax. Thus packed the virus remained active for 8 days. Edwards remarks that "the original richness of the injected blood in virus is, however, a factor of considerable importance and sometimes the period of survival for this reason is likely to prove disappointing." Although it is not clear how the quantity of the virus influences the keeping quality, it appears from this remark that even under these conditions of despatch the virus did not always survive for 8 days.

The serum-virus method may set up new centres of infection by killing or causing severe reactions in inoculated animals and Cooper (1931b) showed that even cattle having a "blocked-out" reaction are capable of transmitting infection. Walker (1929) considered that cattle failing to react may contract rinderpest from reacting cattle. Since Edwards has shown that, provided active virus is used, non-reactors are immunized, failure to protect susceptible cattle must be due to the use of inactive virus.

In some countries the losses caused by protozoa injected with the virus at times reached 20 per cent. Daubney (1928) mentioned the loss of 50 per cent. from Babesidae in 18 cattle whose resistance to this parasite had been greatly reduced by short interval dipping. In India the serum-virus method is apt to cause a flare up of latent protozoa.

Although von Ostertag (1916) considered that in countries where protozoal diseases are endemic the cattle inoculated would be immune to those diseases and therefore the danger of spreading them could be disregarded, nearly all workers using the serum-virus method have found it advisable either to kill the protozoa in the virus or when necessary to treat animals after inoculation. Where large numbers of animals are dealt with, the disadvantages of post-injection treatment are obvious. Trypanosomes in the blood may be destroyed by tartar emetic (Carmichael 1926), but it has been pointed out that this destroys *T. vivax* but not *T. congolense*. Trypan blue is used to destroy Babesidae but instead of adding the drug to the blood after withdrawal it may be injected intravenously to the virus doner the day before bleeding (Conti 1933). Even though the virus is freed from contaminants it is apt to cause a flare up of latent infections and for this reason the serum-virus method remains one requiring observation and treatment of inoculated cattle.

To exclude protozoan contaminants, virus production in other animals was investigated. That sheep were susceptible was noted by Galambos in 1861 and later by Koch (1897), Rogers (1900) and Nicolle and Adil Bey (1902). Use was made of sheep by von Ostertag (1916) and Walker and Bradshaw (1925) who observed that they were not susceptible to the piroplasmoses of cattle.

Edwards in India successfully used goats to cleanse the blood of protozoa, with what far reaching results will be detailed later. Haddow, to overcome the rapid inactivation of the virus in blood, sent it out in the form of spleen pulp. In other countries goats have been tried with disappointing results (Kearney 1930).

The evidence on the duration of the immunity conferred by the serum-virus method is somewhat contradictory. Some regard it as life long, but Daubney (1930) is probably nearest the truth when he says that in animals guarded against all risks of reinfection the immunity begins to decline two to three years after the serum-virus inoculation, and that long immunity is due to symptomless reinfection.

Banerji (1933) and D'Costa and Balwant Singh (1933b) have shown that cattle virus and serum confers a longer immunity than goat virus and serum.

In Turkey the serum-virus method has been displaced by vaccine. Tahsin and Richter (1934) and Delpy (1935a) hold that it is too expensive and the losses caused by it too heavy. Nevertheless many still consider the serum-virus method to be the best. That it is still widely used even in countries where vaccine has been employed, is shown by the fact that of 173,881 animals immunized in Kenya in 1934, 98,373 received serum-virus (Brassey-Edwards 1936).

(e) *Vaccines prepared from Killed or Attenuated Virus.*

To overcome the disadvantages inherent in the serum-virus method of immunization, a vast amount of work has been done on the production of a vaccine from the virus killed or attenuated by exposure to chemicals, such as glycerine, formalin, phenol, toluol, and chloroform, either alone or in combination, one chemical being used to attenuate or kill the virus and the second added to preserve the vaccine.

The virus in the blood proving unsatisfactory for the preparation of a vaccine, various organs were examined, and as in the earlier work somewhat large doses of vaccine were found necessary, the practice was to incorporate in the vaccine as many organs as possible, mainly with the idea of increasing the bulk and hence the number of doses from each animal. Gradually the tendency grew to exclude the liver and other organs of low antigenic value and those liable to contamination, such as the mesenteric lymph glands, so that to-day many favour using only spleen. The aim of the research worker has been to prepare easily and quickly at low cost, a vaccine which would not transmit rinderpest or other infections nor cause undue local reaction, yet would immunize rapidly and for a long time and retain its immunizing properties when exposed to external temperature. After briefly reviewing the earlier work, the vaccines more commonly used to-day will be summarised in the light of these desiderata.

The satisfactory results with heated blood recorded by Gordziaklowski (1921) were not confirmed. Daubney (1928), Beaton (1931) and Hall (1933) failed to immunize with blood.

Some of the earliest work on the preparation of a vaccine was that of Kakizaki (1918) and his co-workers, who attenuated the virus with glycerine and added toluol and other preservatives. The exceptionally good results reported were queried by some investigators; but were later in part confirmed by Bennett (1936), who pointed out that Kakizaki used glycerine-water, whereas others had apparently used glycerine-saline.

In 1917 Boynton detailed the progress of his work, and in 1928 described the preparation of the vaccine finally evolved. He heated for three hours at 44° C. the minced lymph glands, spleen, liver, heart, kidneys and testicles to which had been added one-third of the weight of glycerine containing 0·5 per cent. phenol. Ageing or attenuation of the vaccine was effected by storage in the refrigerator for from one to six months. As the immunizing properties of the vaccine, which had to be determined, were lost soon after it was sufficiently attenuated, the material had to be used soon after the vaccine stage' had been attained, and because of the poor keeping qualities no surplus stock could be kept on hand for use in an emergency. Some of the organs, notably the liver, were added simply to make up bulk and not for their antigenic properties. Boynton considered that the potency of the vaccine could be varied by the time and temperature at which it was stored. By killing the virus with chloroform, Kelser (1927) and Kelser *et al.* (1927) sought to overcome the drawback of having to attenuate the vaccine by storage and subsequently having to test it at intervals for attenuation and potency. Allowing for the preparation of the pulp, and the storage to overcome the irritant effects of chloroform on subcutaneous injection, Kelser's vaccine was ready for use after three days. The spleen, liver, kidneys, testicles and various lymphatic glands were used. The dose given was three injections of roughly ten grams at weekly intervals. Rodier (1928) further altered the vaccine by using only spleen, lymph glands and tonsils, and giving only one injection. Kelser considered that cattle could be protected by a single dose of his vaccine, but that the more susceptible buffaloes required three or more injections; while Rodier, according to Kelser, showed that a single dose of vaccine prepared from lymph glands alone immunized buffaloes, and because of their greater susceptibility these animals were given half the dose necessary for cattle. This seems to indicate that Rodier was using an attenuated and not a dead virus.

The value of formalin in the preparation of rinderpest vaccine has been investigated by a number of workers, the earliest of whom appear to be Curasson and Delpy (1926), and Daubney (1928).

The work on Foot-and-Mouth Disease published by Vallée, Carré and Rinjard in 1926 induced Curasson and Delpy (1926) to use formalin to kill the virus of rinderpest and they produced a vaccine by submitting spleen pulp to the action of 1/250 formalin in normal saline for 48 hours at room temperature in the dark.

At the same time Daubney (1928) also influenced by the work of Vallée and his co-workers and by the reports of Bedson, Maitland and Burbury (1927), was investigating the value of formalinized spleen as a vaccine against rinderpest. He carried out extensive investigations and dealt with various aspects of the subject. His vaccine was prepared by mincing in a Latapie the spleen removed from infected animals bled to death under chloroform anaesthesia on the seventh day after inoculation with virulent blood. The pulp was mixed with Tyrode's solution containing 1/1,500 formalin, and allowed to stand for 72 hours at 5° C. The vaccine was given in doses of one gram and was said to retain its potency for seven days at 5° C. and for five days at room temperature. Daubney remarked that the preservation of antigenic properties was greatly influenced by the pH value of the vaccine. The vaccine was safe but to be effective had to be used soon after preparation.

Many publications have appeared on the subject of formalinized tissue vaccine. Investigators have varied slightly the concentration of formalin used and the period and conditions of storage. A solution of 0·01 per cent. formalin appears to attenuate the virus more rapidly than exposure to bodyheat, so that up to 97° F. the conditions of storage do not influence the rate of attenuation. Formalinized spleen pulp has been employed in doses of 1 gram (Daubney 1928); 8 grams (Hall 1930); and 20 grams (Jacotot 1929).

To increase the value of formalinized vaccine Prunier (1931) added castor oil and Curasson (1931) added tapioca.

Bennett (1936), following on the work of Kakizaki, prepared a vaccine by adding double the weight of 60 per cent. glycerine-water to minced spleens, the spleen being removed " from cattle plague patients at the height of an acute febrile reaction ", and storing for three days at 37° C. or for three months at 0° C. The vaccine retained its immunizing properties for one month at 37° C. and for one year at 0° C., and in doses of one gram spleen pulp per 100 kilograms was claimed to immunize for about eight months, the immunity being developed within three days. The temperature of storage is important, as it is the temperature and not the glycerine which is said to attenuate the virus.

Boynton (1928) stressed the deleterious effect of impure glycerine, Daubney (1928) noted the deterioration of formalin by polymerization, and Walker (1929), who remarked on the variability of the antigenic value of spleens, advised that for vaccine preparation several spleens should be pooled and tested for potency before use.

Dried vaccines.—Andrievsky (1931) immunized cattle with a vaccine prepared from dried spleen and glands emulsified in formalin-saline. Curasson (1931) dried the tissues in vacuo over sulphuric acid and after storing at room temperature for a month the material was used in doses of 2, 4, 6 and 8 grams. Jacotot (1931) used dried spleen stored for a month at room temperature, and this in doses of one gram, without the addition of antiseptic, gave immunity in one month. Robles and Generoso (1930), working in the Philippine Islands, prepared a dried rinderpest vaccine from the pulp of spleen, lymph glands and tonsils by storing at 0° to 8° C. in a desiccator over calcium chloride. After 3 to 5 weeks drying it was used as a vaccine in doses of 1 gram. The method of washing and mincing was somewhat lengthy and tedious and the vaccine took at least three weeks to prepare. After 84 to 91 days in the ice chest it conferred only a very low degree of immunity, and became avirulent in less than seven days at room temperature. They say it is " impractical to send vaccine in fragment form to the field, since its preparation for use would necessarily be done under adverse field conditions involving too great a danger of contamination ".

In a second article (1931) the same workers record further progress and state that delay in drying seems to destroy potency. They claim that powdered rinderpest vaccine kept in the ice chest for 275 days suffered no loss in potency and that 30 days at room temperature did not destroy its immunizing powers, but that delayed drying appeared to be harmful to the immunizing value of the vaccine.

To prevent abscess formation formalin has been incorporated in dried vaccines, either before drying or when preparing the powder for injection. Andrievsky (1931) exposes dried spleen, tonsils and lymph glands to the action of formalin water for 48 hours immediately before injection. Delpy (1935a) prepares formalin vaccine and strains it through a coarse cloth, using both the filtrate and the solid residue which is first dried in electric stoves. 0·5 gram of the dried vaccine immunizes in 12 days.

The safety of vaccines, the retention of immunizing properties, the time taken to confer immunity, and the duration of immunity are of great practical importance.

It is claimed for all tissue vaccines that they are safe in that they do not transmit protozoa or other blood parasites. It is claimed, too, that unlike the serum-virus inoculation, tissue vaccines do not cause severe reactions and so do not set up fresh centres of infection. Such claims should, however, be taken to refer only to the cattle on which the work was carried out and to the countries in which the work was done. It is conceivable that a vaccine harmless for fairly resistant cattle might prove dangerous for more susceptible animals, and there is evidence that this is the case. Daubney's formalin vaccine was perfectly safe immediately after preparation when used in East Africa on the cattle of that country, but Cooper (1931b) at Muktesar found that even four days after preparation it was still highly infective for hill cattle. In Indo-China formalin vaccine is used as it does not spread the disease (le Louet 1932), and there is no record from any other country of tissue vaccine spreading rinderpest. Although some vaccines are given in doses up to 60 c.cms. the local reaction causes no alarm, but vaccines containing chloroform and toluol are apt to cause oedema and abscess formation (Andrievsky 1931, and Granonillit and Do-van-Vien 1935).

Formalinized vaccine is said to retain its immunizing properties at room temperature for one month (Juge 1935); for one month even in the tropics (Curasson 1934); for five weeks (Missenard and Zylbertal 1934); for two months (Delpy 1935a); for ten months (Ismail and Zudhi 1933); for two months at 30° C. (Jacotot 1932); and when dried for six months (Jacotot 1932); and for one year (Delpy 1935a).

Immunity is established in 4 to 8 days (Jacotot 1932); 8 days (Curasson 1930, Ismail and Zudhi 1933, Delpy 1935b); 4 to 11 days (Daubney 1928); 10 to 12 days (Curasson 1936); 10 to 12 days in laboratory experiments and 20 days under field conditions (Juge 1935); 12 days (Delpy 1935a); and 15 days (Jacotot 1929).

To accelerate the onset of immunity the vaccine has been given intradermally, it being claimed that by this method the period is reduced to four days (Curasson and Zylbertal 1934, Ismail and Zudhi 1933). The latter authors recommend that 5 c.cms. of the vaccine be given intradermally into the the scrotum or lips of the vulva. Curasson (1930) finds that in infected herds, where the vaccine cannot be given subcutaneously, 10 c.cms. if given intradermally, produces immunity in 4 days. Jacotot and le Roux (1935), however, have found no advantage in giving the vaccine intradermally.

The duration of the immunity is said to be four months (Juge 1935); five to six months (Missenard and Zylbertal 1934); six months (Jacotot 1932, Curasson 1936); seven months (Ismail and Zudhi 1933); twelve months (Curasson 1930, Delpy 1935a); about two years, and five years when the vaccine is followed by active virus (Daubney 1934).

Bennett's glycerine vaccine retains its immunizing properties for thirty days at 37° C. and for one year at 0° C., and immunizes for eight months, the immunity being serviceable for all cattle and solid for most within three days, and solid for all in one week.

The cost of the vaccine is not easy to determine, but where the vaccine is prepared at the scene of the outbreak from a sick animal it is negligible. In Persia one calf produces about 200 doses of vaccine, at which rate it is considered cheap, and because of the simplicity of manufacture it is used in that country (Delpy 1935a). Curasson (1936), however, considers that the cost of preparing formalin vaccine in a central laboratory would be prohibitive, and for that reason prepares it at the scene of the outbreak.

Obviously there is no great unanimity in the findings of those who have worked on tissue vaccines. It would seem that Bennett's glycerine vaccine is not influenced by the diluent, the rate of attenuation depending solely upon the temperature at which it is kept. All other chemically treated vaccines depend upon the action of the agent, and the best in a dose of 1 gram per 100 kilograms confer an immunity lasting little more than twelve months established in not less than one week. This refers only to fresh vaccine. Bennett's glycerine vaccine in a dose of 1 gram per 100 kilograms in three days confers an immunity which lasts somewhat less than a year. This also refers to vaccine stored at a low temperature as although Bennett claims that even after storage for 30 days at 37° C. the vaccine " produces a very serviceable immunity in all cattle and a solid immunity in most cattle within three days ", his protocols do not justify this. The immunity of only two cattle given vaccine stored for 3 days at 37° C. was tested 3 days after the injection of 1 gram per 100 kilograms and of these one died of rinderpest and the other had diarrhoea and ulcers in the mouth.

Daubney (1934) obtained excellent results by giving partially inactivated vaccine seven days after completely inactivated vaccine. He concluded that active or partially active spleen virus given after completely inactivated vaccine confers an immunity serviceable for more than five years. This method of immunization has at least some of the disadvantages associated with the use of virus in the serum-virus method.

Although tissue vaccines are now widely used, in assessing their value one should not lose sight of the fact that such a careful worker as Edwards (1927b) could not obtain satisfactory results with them, and at Muktesar this line of research was finally abandoned in 1932 after Haddow had found that formalinized vaccine was apt to cause fatal reactions and, when it was safe, the immunity conferred was unreliable. Moreover Mettam (1934) records that field results with formalin vaccine were so unsatisfactory that further experiments were carried out with other chemicals. He apparently had encouraging laboratory results with the addition of 1 per cent. sodium tauroglycocholate, normal saline, and 1/1,000 formalin to spleen pulp finely ground up with sand. One finds also that in 1934 in Kenya, where Daubney and others did a great deal of work on the production of formalinized tissue vaccine, of 173,881 cattle which received prophylactic rinderpest treatment, 65,607 received vaccine alone, 9,029 were given vaccine plus virus, 98,373 were given serum plus virus, and 872 received serum alone (Brassey-Edwards 1936).

(*f*) *Goat adapted virus.*

Apparently the earliest record of rinderpest in the goat was that of Jessen in 1836 (Flemming 1882). Others who drew attention to the susceptibility of this species were Chicoli (1863), Hallen (1871), and Topacio (1926). Koch (1897) observed no change in the virus maintained in sheep, but noted some attenuation after five passages in goats, although even then the virus killed the susceptible South African cattle. Hall (1933) effected no attenuation after 27 passages in goats. Saunders and Ayyar (1936) showed that the virus attained a sufficient degree of attenuation for use as a vaccine only after about eighty passages in the goat. To fix the virus in goats Edwards (1927a) found it necessary to introduce " the bovine virus, by careful surgical operation, on the foetal side of the foetal envelopes in the pregnant goat ". Other workers have not had to resort to this. Hall (1933) could not maintain cattle virus in goats, losing one strain after 14 passages and another after 27 passages. To Edwards belongs the credit of realising the value of the goat for attenuating the virus for immunizing cattle. He in the first instance successfully used goats to overcome one of the disadvantages of the serum-virus method of immunization, namely, the injection of protozoa with the virus. Edwards reported on the value of goat virus alone, but did not recommend the entire abandonment of the serum-virus method, advising instead the replacement of serum alone by goat virus plus serum, and Cooper (1931), after showing that goat virus alone could be used, did not recommend it in view of the grave risks involved " for a tract in which the animals are considerably more susceptible is liable to be encountered suddenly at any time ". It should, however, be noted that according to Banerji (1933) Bliss in 1922 had recommended the use of goat virus in China, and in 1926 Topacio had pointed out the economy of this method.

Edwards (1930) observed at Karnal the survival of three bulls, which had been injected with goat blood virus alone by his colleague, Mr. Menon. Stirling (1932) noted this and also became acquainted with the fortunate error of his assistant, Mr. P. S. Nair, who while doing serum-virus inoculations gave five cattle goat virus and then found his supply of serum exhausted. All five cattle lived. These two cases and encouragement from Edwards led Stirling to carry out a small experiment with goat virus alone, and it is of interest to note that the four cattle on which the experiment was tried were obtained on loan from an Indian villager, after he " had been induced to make a written statement that the experiments would be carried out at his risk ". This small experiment was followed by the injection of 343 cattle and buffaloes, all of which survived the subsequent injection of bull virus. These appear to be the first experiments on the use of goat virus recorded in India.

Edwards (1927a) recommended field workers to prepare their own virus by the inoculation of two goats with Muktesar virus. Kerr and Menon (1934) investigated the value of the virus for buffaloes and for use in outbreaks. Stirling 1933 remarked on the value of the virus for cattle " with a relatively high degree of natural resistance " but still recommended the well-known serum-virus method for immunizing highly susceptible cattle in India and other countries. Stirling's method of preparing the virus was to bleed the infected goat on the fourth or fifth day. He considered female goats unsatisfactory as virus producers on account of the liability of pregnant goats to abort. Almost immediately workers were confronted with their first difficulty, which was to get active virus to the scene of inoculation. Kerr and Menon (1934) attempted to prepare virus in the field by infecting virus producers at the scene of inoculation, but discarded this method because the technique was beyond the ability of field inoculators. They, therefore, concentrated on the use of spleen tissue vaccine, the spleen of reacting goats being cut into strips of about one gram each, and emulsified in saline immediately before injection by grinding in a mortar for half an hour, specially large needles being required for the inoculation. One goat produced between 2,000 and 2,500 doses, the dose being 0·01 gram of spleen. They injected large numbers of buffaloes and noted severe reactions only in those worked immediately after inoculation. To modify this the dose for buffaloes was reduced to half that for cattle. But even the use of spleen tissue did not overcome the limited viability of the virus, and Kerr (1935) records failures on two occasions due to the use of inactivated virus. One was due to the virus being used more than seven days after issue, and the other was during the very hot month of April. He noted specially that if his dose of 0·01 gram for cattle could be halved, there would be a very big saving. Kerr considered that increasing the concentration of the spleen to above one per cent. retarded or prevented immunization "probably due to the more ready diffusion of the thinner emulsion ". He also considered that the virus lived longer in a saline solution weaker than normal saline, and finally used a half per cent. solution of sodium chloride for emulsifying his spleen tissue: Haddow (1936) found that the virus in tissue and tissue extracts remained active for 3 to 6 weeks at room temperature but for nothing like this at incubator temperature or even at 30° C. Haddow (1937) mentions that the rapid loss of viability of virus in hot weather is still " a cause of inconvenience " and adds that at room temperature the virus in spleen is viable for about a month but extracted with distilled water or normal saline becomes avirulent in about a week. As the temperature is such an important factor in the viability of the virus, it is unfortunate that the room temperature is not stated. Muktesar, at an elevation of about 7,000 feet, is comparatively cold, the variation in temperature being given by Lingard (1905) as from below 32° F. to 86° F. in the shade, and Ware (1933) states that " The climate of the place is for the most part of the year cold and the extreme temperatures range between 17·9° and 87·4° F." The time of the year, therefore, has an important bearing on Haddow's observation.

Haddow (1933) considers goat virus useful in imported animals, but in indigenous animals the virus resuscitates latent infection, which is as serious as conveyed transmission. The duration of the immunity conferred by goat virus vaccine is put at twelve months (Stirling 1933, Kerr 1935); and at something under three years (Edwards 1927a). On the time taken to develop immunity there is very little evidence—Stirling (1933) puts it at " about a week ".

Vaccines made from cattle virus are safe only if the virus has been attenuated. Vaccines made from goat virus are effective only if the virus is fully potent. In spleen tissue goat virus is said to be viable for 30 days at 45° F. (Kerr and Menon 1934, Kerr 1935); for 6 days at room temperature (Kerr 1935); and for seven days at room temperature up to 85° F. (Kerr and Menon 1934). Haddow (1934) found that " at ordinary temperatures " at Muktesar spleen tissues and watery extracts of spleen tissues remained virulent for about a month, and seemed to think that under hot weather conditions on the plains, the virus remained virulent for 15 days. Since Kerr and Menon (1934) failed to immunize with vaccine exposed to the plains temperature for six days, Haddow seems unduly optimistic.

III. EXPERIMENTAL WORK CARRIED OUT AT INSEIN.

A study of the literature on rinderpest was made during leave spent at Onderstepoort Laboratory, South Africa, in 1934 and again in 1937, and on my return to Insein in June, 1934, work was commenced by attempting to fix the virus neurotropically in white mice. At the same time the vaccine value of an emulsion of goat spleen in glycerine-saline was examined, and to determine the effect of storage on cattle virus the spleens of cattle were removed at the height of the thermal reaction and stored at 42° F. for further examination.

Attempts to fix the virus in mice on the lines of the work of Max Theiler (1930), Sawyer and Lloyd (1931) and Alexander (1933) were unsuccessful, and as the results with glycerine-saline emulsion of goat spleen were unsatisfactory, work on this was soon abandoned. Storage of cattle virus gave promising results and in March, 1935, it was possible to report to the Director of Veterinary Services, Burma, "there are reasons to believe, from the work done here, that simple storage will attenuate cattle virus sufficiently for it to be used as a vaccine." On the failure of glycerine-saline emulsion of goat spleen, work had in the meantime been commenced on a vaccine prepared from dried goat spleen. The results were so good that the efforts were concentrated on this aspect of the subject, and all other investigations on rinderpest discontinued. In March, 1938, however, a small experiment was done with Bennett's glycerine vaccine.

All experimental work was conducted at the Veterinary Research Laboratory, Insein, which is situated in the midst of a thickly populated area about eight miles from the centre of Rangoon. The grazing grounds, about fifteen acres in extent, provide grazing for less than six months in the year, and are used for all experimental animals. The cost of feeding is, therefore, high, and represents a large percentage of the very small amount granted for Veterinary Research. Under these circumstances experiments can be undertaken on only a very small scale and full use must be made of experimental animals. The experimental work was carried out as funds, animal accommodation, seasons, and numerous other duties would permit.

Rinderpest in goats.—In the earlier experiments, goats were obtained from the neighbourhood of the Laboratory, but with the use of large numbers it was necessary to get them from Upper Burma. As there appears to be no variation in the susceptibility of goats in Burma, in the experimental data their origin is not stated.

Rinderpest in goats has been described by Edwards (1927a), Stirling (1932), Beaton (1930), Cooper (1931), Hall (1933), D'Costa and Balwant Singh (1933), and Saunders and Ayyar (1936) Hall was unable to maintain cattle virus by passaging in goats, and thought if goats were to be used for virus production they would need to be specially bred and kept free from infection. D'Costa and Balwant Singh observed a breed susceptibility and noted that the mortality, which varied with the season and the virulence of the virus, was from 70 per cent. to 100 per cent. Stirling described a jugular pulse, which, in his view, was all that need be looked for in a virus producing animal. He noted a mortality of only 15 per cent.

The lung lesion, which Hall (1933) does not consider a constant feature, has led to the disease being confused with contagious pleuropneumonia. Saunders and Ayyar (1936) passaged cattle virus 150 times through 300 goats and noted no change in the reaction in goats. Attenuation for cattle did not occur until after the 80th passage.

No natural infection in goats has been observed by me. At Insein the Muktesar virus has been passaged 302 times in 811 goats, obtained from widely scattered areas in Burma. No change in the reaction has been observed and the lung lesion has been a constant feature. Of the 811 goats, 23 died on the fourth day and all these had bronchopneumonia. As all the goats are temperatured for at least three weeks before being used for rinderpest purposes, and none are put into an experiment unless they have run a normal temperature for at least seven days, it seems likely that the pneumonia was that commonly associated with rinderpest. Small patches of early bronchopneumonia have been seen in several goats slaughtered for vaccine production on the 4th day after infection. In goats slaughtered for vaccine production there is always an enlargement of the spleen, which is due to the engorgement with blood and hypertrophy of the lymphoid follicles.

Of 162 goats in which the disease was allowed to run its course, 86 showed the initial rise in temperature on the 1st day after infection; 58 on the 2nd day; and 18 on the 3rd day. The peak temperature was reached in 3 animals on the 1st day; in 10 on the 2nd day; in 52 on the 3rd day; in 50 on the 4th day; in 16 on the 5th day; in 14 on the 6th day; in 10 on the 7th day; and in 7 of the 8th day. The rise in temperature after injection of the virus was between 1° and 1·9° F. in 9 goats; between 2° and 2·9° F. in 18 goats; between 3° and 3·9° F. in 67 goats; between 4° and 4·9° F. in 55 goats; and between 5° and 5·6° in 13 goats. Three goats showed an indefinite temperature reaction, and four reacted but survived. The other 155 died, the deaths occurring as follows:—4 on the 3rd day after infection, 9 on the 4th day, 13 on the 5th day, 13 on the 6th day, 20 on the 7th day, 15 on the 8th day, 19 on the 9th day. 11 on the 10th day, 9 on the 11th day, 2 on the 12th day, 2 on the 13th day, 5 on the 14th day, 1 on the 15th day, 3 on the 16th day, 2 on the 17th day, 2 on the 18th day, 1 on the 20th day, and 27 between the 21st and 86th day. Those which lived for some time became very emaciated and had extensive lesions of bronchopneumonia.

The cattle used in experiments.—In their resistance to rinderpest the cattle of Burma vary almost directly with the period that the areas from which they come have been free from the disease. In these experiments the term " Local cattle " refers to cattle bought on the Rangoon market, which is supplied from all parts of Burma. " Kyaukpyu cattle " are obtained from the Island of Ramree, off the west coast of Burma, and have been conveniently called " Kyaukpyu cattle " as they are purchased not far from Kyaukpyu and shipped from that port. They are small animals, seldom more than two hundred pounds in weight, and very susceptible to rinderpest. In appearance and susceptibility to rinderpest they resemble the hill cattle used at the Muktesar Laboratory.

(*a*) *Experiments* 1 *and* 2 *with cattle virus.*

Experiment 1, *Table* 1.—*The passage of cattle virus in susceptible cattle.*—In all experiments from April, 1935, involving cattle virus for the testing of immunity or for any other purpose, the Kungyangon strain of virus was used. This was obtained from a natural outbreak of the disease in cattle in the Kungyangon village. It has been kept going on Kyaukpyu cattle which, as has been mentioned earlier on, have very little resistance to rinderpest. From Table 1, which gives details of the reactions of the cattle used for maintaining this virus, it will be noted that:—

(i) The initial rise in temperature was shown by—
  16 cattle on the 2nd day,
  39 cattle on the 3rd day,
  4 cattle on the 4th day.

(ii) In the 18 cattle in which the disease was allowed to run its course, the peak temperature was reached—
  on the 4th day by 6 cattle,
  on the 5th day by 5 cattle,
  on the 6th day by 5 cattle,
  on the 8th day by 2 cattle.

(iii) The rise in temperature after injection of the virus was—
  between 2° and 3° F. in 3 cattle,
  between 3·1° and 4° F. in 25 cattle,
  between 4·1° and 5° F. in 22 cattle,
  between 5·1° and 6° F. in 9 cattle.
  The figures include animals slaughtered on the 5th day.

(iv) Of 59 cattle—
  1 recovered,
  17 died,
  38 were destroyed for conservation of the virus.

(v) Of the 18 animals in which the disease was allowed to run its course, 17, or nearly 95 per cent., died. These figures should be borne in mind when assessing the value of immunity tests in experiments to be described later.

Experiment 2, Table 2.—The effect of storage at 42° F. on cattle virus in the form of powdered spleen dried in vacuo over calcium chloride.—In this experiment two strains of cattle virus had to be used. Table 2 shows that after 100 days' storage the virus killed both animals injected, after 133 days' storage it killed one of two cattle, and after 177 days it killed none of the three animals injected. All the animals which survived the virus subsequently proved immune to fresh virulent blood. From this it seems that storage at

42° F. for about six months renders the virus safe and effective as a vaccine for susceptible cattle. Bennett (1936) found that spleen pulp in glycerine-water could be used as a vaccine after three months' storage at 0° C.

(*b*) *Experiments 3 to 7 with goat adapted rinderpest virus in the form of an emulsion of spleen pulp in glycerine-saline.*

In all these experiments by " glycerine-saline " is meant equal volumes of best quality glycerine and normal saline, and in all cases the spleen was removed from a goat on the fourth day after injection with rinderpest virus.

Experiment 3, *Table* 3.—*The viability at* 42° *F.*—The viability at 42° F. was determined on goats, the limit of the test being 231 days, at which period the virus was still viable. Virus stored up to 80 days provoked a rise in temperature which reached its peak on the 3rd, 4th or 5th day. After 102 days' storage both the initial rise in temperature and the peak temperature occurred later.

Experiment 4, *Table* 4.—*The viability at* 83° *to* 86° *F.*—The viability at 83° to 86° F. was tested after 3, 6 and 9 days' storage. All the goats reacted but although the initial rise occurred early the peak temperature was delayed, and this, and the fact that no goats died, seemed to indicate that at 83° to 86° F. glycerine-saline rapidly attenuated the virus. At this stage the use of goats as experimental animals was discontinued.

Experiment 5, *Table* 5.—*The immunizing properties of varying quantities of a* 4 *per cent. emulsion of spleen.*—The more resistant local cattle were used, and given doses of 1, 2, 3 and 4 c.cms. Of the three animals which gave no reaction to the vaccine, one died and two reacted very severely to the virus. Of the other eight cattle, which developed mild but delayed temperature reactions to the vaccine, virulent blood killed one, caused a severe reaction in four, a mild reaction in one, and had no effect on two. Four c.cms. of 4 per cent. emulsion of spleen gave no more protection than a quarter of that quantity. These results were unsatisfactory, but in the meantime experiment number 6 had been started.

Experiment 6, *Table* 6.—*The duration of immunity conferred by an emulsion of spleen pulp in glycerine-saline.*—Local and Kyaukpyu cattle were used. It was originally intended to test the immunity of a few animals at intervals over a period of two years, but after the unsatisfactory results obtained at the first immunity test another lot was tested immediately. No reaction to goat virus indicates either immunity to rinderpest or no protection from the goat virus. In other words, unless goat virus provokes a temperture reaction it fails to immunize. On this basis numbers 267 and 275 of the six cattle given vaccine stored for ten days at 42° F. may be disregarded. Of the other four in this group one gave a good temperature reaction to the vaccine and did not react to the virus, and three were killed by the virus. Of eight cattle given vaccine stored for 2 days at 42° F. and two days at 84° to 86° F., the virus killed three and produced severe reactions in five. Vaccine stored for 2 days at 42° F. and 8 days at 84° to 86° F. protected one out of eight animals. The other seven were killed by the virus.

In addition to the 22 animals detailed in Table 6, ten other cattle were inoculated in this experiment. Two and a half months after they had been given vaccine, rinderpest broke out amongst them and killed seven. Before finally abandoning this line of investigation a last experiment was done.

Experiment 7, Table 7.—The immunizing properties of (*a*) 1 *per cent. emulsion in* 50 *per cent. glycerine-saline,* (*b*) 1 *per cent. emulsion in normal saline, after storage for* (i) *Five days at* 42° *F.* (ii) *Five days at* 82° *F.*—In this experiment the more resistant local cattle as well as the susceptible Kyaukpyu cattle were used. After five days at 42° F. both emulsions provoked temperature reactions, but only two of the eight animals showed no reaction to the subsequent injection of virulent blood. The vaccine stored for five days at 82° F. caused a temperature reaction in seven of the eight cattle. The eighth animal gave no reaction to virulent blood, so was presumably immune when it was given the vaccine. Of the seven reacting animals, four gave no reaction to virulent blood, two gave mild temperature reactions, and one reacted very severely.

The results obtained in these experiments showed that an emulsion of goat spleen in normal saline or in glycerine-saline had little protective value. It is possible that during the preparation of the vaccine the virus was attenuated, but careful checking up has failed to reveal any likely source of error. The goats which supplied the spleens also supplied blood for the passage of the virus, and for this purpose were bled immediately before being killed for removal of the spleen; so it may be concluded that the spleen on removal from the goat contained virulent virus. Walker (1929) pointed out the low antigenic value of certain spleens, but as three different spleens were used in these three experiments, it is unlikely that all three were low in antigenic value. Boynton (1928) remarked on the necessity of using only good quality glycerine in the preparation of rinderpest vaccine, as poor quality glycerine causes rapid deterioration of the virus. Although only glycerine of the best quality was used, it might have been affected by this tropical climate. It is, possible, however, that the virus, greatly attenuated for cattle by passage through goats, is still further weakened by glycerine. Whatever the reason, these experiments rule out the possibility of a satisfactory vaccine being prepared in this manner for use in Burma. Attention was therefore directed to the properties of virus in the form of dried spleen.

(*c*) *Experiments* 8 *to* 17 *with goat adapted rinderpest virus in the form of spleen powder dried in vacuo over calcium chloride.*

The goats supplying the spleen for use in these experiments were killed on the fourth day after injection with virus. The spleens were immediately minced in a Latapie and the pulp dried in a desiccator, in which it was kept at 42° F. for 24 hours. For each experiment the pooled material from not less than three spleens was used.

Experiment 8, *Table* 8.—*The viability at* 42° *F.*—The viability at 42° F. was determined at varying intervals up to 231 days. Disregarding for the moment the goat given material stored for 192 days, the reactions from virus stored up to 141 days were very similar to those provoked by fresh virus; the initial rise occurred on about the 3rd day and the peak temperature was reached on the 5th or 6th day. After 141 days' storage the initial rise was delayed and the peak temperature was reached much later. This delayed temperature reaction may be interpreted as indicating attenuation after about five months' storage. Similar results at the same temperature were obtained with goat virus in an emulsion of glycerine-saline, though in this case attenuation seemed to occur in less than four months, while cattle virus in the form of dried spleen became attenuated in just about the same time as dried goat virus (experiment 2). There are, therefore, grounds for thinking that although passage in goats has attenuated the virus for cattle, it has not changed its reaction to storage at 42° F.

Experiment 9, *Table* 9.—*The viability at* 85° *to* 89° *F.*—This was determined on goats and was unfortunately not carried to its conclusion. As far as it went the experiment showed that the virus survived 13 days at 85° to 89° F., and even after this period the initial rise in temperature and the time taken to reach the peak were well up to the average for fresh virus. Experiment 4 showed that at 83° to 86° F. glycerine-saline rapidly attenuated the virus. The conclusion was drawn that the virus is preserved longer in the form of dried powder than as an emulsion in glycerine-saline, and having arrived at this conclusion it was decided to investigate the immunizing properties of dried virus, and in this and all future experiments cattle were used.

Experiment 10, *Table* 10.—*The viability and immunizing properties of dried spleen virus emulsified in normal saline.*—Neither four days at 42° F. nor four days at 79° to 81° F.had any deleterious effect on the antigenic properties of the virus emulsified in normal saline. As storage for four days at 79° to 81° F. had no ill effect on the virus it seemed practicable to despatch vaccine in the form of dried spleen to be emulsified with normal saline on the day of injection.

Experiment 11, *Table* 11.—*The immunizing properties of dried spleen virus after storage for* (i) 5 *days at* 42° *F.*, (ii) 5 *days at* 82° *F.*—For each period of storage two Kyaukpyu and two local cattle were used, each receiving 0·005 gram of dried spleen. All reacted to the virus and all proved immune to virulent cattle virus. As these two small experiments had shown the undoubted antigenic properties of dried spleen, emulsified in normal saline, another small experiment was carried out to determine the effect of exposure to a temperature of 97° F.

Experiment 12, *Table* 12.—*The effect of storage at* 97° *F. on the viability and antigenic properties.*—Three cattle were given virus stored for 3 days and 3 cattle were given virus stored for 6 days. Three days at 97° F. had no deleterious effect on the virus, but after 6 days at 97° F. the effect was marked, only one of three cattle being immunized. Experiments had now shown that very small quantities of the virus immunized and that a temperature of 97° F. rapidly destroyed the antigenic properties of the virus. Many parcels of vaccine despatched to the field would for hours be exposed to a temperature of 97° F. or higher, and as it had been shown that after 6 days at 97° F. the vaccine was unreliable as an immunizing agent there seemed to be no object in investigating the viability of the vaccine at room temperature, which would be in the neighbourdhaad of 85° F. It seemed more important to gain information on the optimum amount of virus required to confer immunity, and on the possibility of despatching vaccine packed in ice.

Experiment 13, *Table* 13.—*The optimum immunizing dose of dried spleen virus.*—Five pairs of cattle were given quantities of dried spleen decreasing from 0·1 to 0·0005 gram. All ten animals were immunized so that it was concluded that 0·0005 gram of dried spleen was sufficient to immunize cattle. Observations, detailed elsewhere in this paper, had been made on the rate of rise of temperature in ice-containing thermos jars. An experiment was now undertaken to determine the behaviour of the virus under what were considered adverse field conditions.

Experiment 14, *Table* 14.—*The effect of adverse field conditions on dried spleen virus.*—To reproduce adverse field conditions virus was packed in a thermos jar of ice and placed in an incubator at 97° F. Three days later the ice was replenished. The jar was replaced in the incubator, and the virus tested at intervals commencing with the sixth day, i.e., nine days after the thermos jar was first placed in the incubator. This procedure was adopted because most of the vaccine would be used later than three days after despatch from the Laboratory. More animals than necessary were put into this experiment, which was regarded as of considerable practical importance, because as it was desirable to use the same virus on all animals injections had to be continued until there was no doubt that the end point had been reached. The results show that six days after the addition of ice to the thermos jar the vaccine immunized all four cattle. After eight days, of four animals one was immunized, one was given no protection, and the other two were given some degree of immunity. After ten days, one of four animals was protected. Observations showed that in thermos jars containing ice and stored at 97° F. the temperature reached 97° F. after four days, so that actually this experiment confirmed the results obtained in experiment 12.

Experiment 15, *Table* 15.—*The duration of immunity.*—Before the optimum dose of vaccine had been determined an experiment to give information on the duration of immunity was commenced and for this purpose a dose of 0·01 gram of dried spleen was given. Local and Kyaukpyu cattle were used and their immunity tested in groups after 319 days, 429 days and 755 days. It should be noted that these animals were protected from exposure to the disease so that whatever immunity they had was conferred by the vaccine, and to be sure that this immunity was not reinforced by unnoticed natural infection, ten to twenty highly susceptible Kyaukpyu cattle were kept with the test herd, it being presumed that these susceptible animals would show the presence of any natural infection.

Of the ten animals in the 319 days group, one developed a rise in temperature commencing on the tenth day, and nine showed no reaction. Unfortunately no contral animals were included in this group, but as the virus was obtained from animal No. 527, Passage No. 45 in experiment 1, and dried spleen from this animal was used to carry on the strain on the 18th November, 1936, it may be presumed that the virus was active.

Of eight animals in the 429 days group, one developed a temperature reaction, the initial rise occurring on the 5th day. One of the other seven animals did not react to the vaccine and all proved immune when given virus.

Of six animals tested after 755 days the two Kyaukpyu animals showed mild temperature reactions, the initial rise occurring on the 6th day in one and on the 7th day in the other. The four local cattle were immune, though one of these did not react to the vaccine and so was probably immune when put into the experiment.

This experiment, which is still in progress, has so far shown that the immunity conferred by the vaccine lasts for twenty-five months.

Experiment 16, *Table* 16.—*The time taken to confer immunity.* —In an experiment carried out in September, 1936, it was shown that immunity was established within three days. A second experiment, done in February, 1938, may be conveniently described as part of the first.

It is hardly possible to summarise the thermal reactions to the vaccine as these were complicated by reactions to the virus. The charts show that virus, given before the temperature caused by the vaccine has returned to normal, provokes a second temperature reaction. Typical charts are shown in Figure 5.

Ten Kyaukpyu cattle were given vaccine and their immunity determined by the injection of cattle virus at intervals varying from twenty-four hours to eleven days atfer the vaccine. Of those tested in September, 1936, three to eleven days after inoculation, one of each pair was given 0·005 gram and the other 0·0025 gram of vaccine. The dose given to those tested in February, 1938, twenty-four to seventy-two hours after vaccination, was 0·0025 gram to one and 0·00025 gram to the other. Both animals in the twenty-four hours group reacted to the virus, one a little more severely than the other, but both recovered. In the thirty-six hours group only one developed mild diarrhoea. None of the animals in the other groups had either diarrhoea or mouth lesions, indicating that 0·00025 gram of dried goat spleen protects against virulent cattle virus injected forty-eight hours later. In the twenty-four hours group the animal which got 0·0025 gram of spleen has a less severe reaction to the virus than the one which got one-tenth that amount, while in the thirty-six hours group the animal with the smaller dose of vaccine reacted to the virus while the one which got the larger dose suffered no ill effects. The experiment shows that 0·00025 gram of dried spleen confers an immunity which is serviceable in twenty-four hours and absolute in forty-eight hours.

Experiment 17, *Table* 17.—*The immunizing value of dried spleen virus stored alternately for twelve hours at* 80° *F. and for twelve hours at* 97° *F.*—Experiment 12 had shown that after 72 hours at 97° F. the immunizing properties of dried spleen virus were unimpaired, and used after 144 hours at this temperature the virus immunized one of three cattle. After 13 days at 85° to 89° F. there was no change in the reaction it produced in goats, though it does not, of course, follow that it would still immunize cattle. It was of practical importance to find out for how long vaccine not kept on ice remained effective during the hot months, and for this purpose during the day it was kept in an incubator and during the night it was kept at room temperature, which did not fall below 80° F.

After storage under these conditions, for six, eight and ten days, the vaccine gave no immunity to one of two cattle in each group, and practically no immunity to the other animal in each group.

Obviously during the hot months vaccine cannot be effectively used six days after removal from cold storage. It seems that the attenuating effect of storage at 97° F. is not arrested by subsequent storage at 80° F.

(*d*) *Experiment* 18 *with Bennett's glycerine-water vaccine.*

The vaccine was prepared exactly as described by Bennett (1936), the spleen being minced in a Latapie pulper.

Experiment 18, *Table* 18.—*The safety and immunizing properties of Bennett's glycerine-water vaccine stored at* 37° *C.*—This experiment was undertaken before recommending this vaccine in the very few outlying areas of Burma where dried spleen vaccine can be used only with difficulty. Bennett found that spleen pulp in glycerine was safe after 3 months at 0° C. My experiment No. 2 showed that after 133 days at 5° C. (42° F.) the dried virus still killed cattle. As observations on Bennett's vaccine stored at 0° C. would take too long, the effect of storage at his second temperature, viz., 37° C., was examined. The vaccine was injected after exposure to 37° C. for four days, six days, fifteen days and twenty days, two cattle being used on each occasion. The Laboratory has no facilities for weighing live cattle. Several Kyaukpyu cattle in Experiment 1 were weighed after they had been slaughtered for removal of the spleen. They all weighed less than 200 lb., and as Bennett recommended a dose of 1 gram spleen pulp per 100 kilograms, all animals in this experiment were given 1 gram of spleen pulp.

Bennett found that his vaccine was safe for use after 3 days at 37° C. and " to produce solid immunity in all cattle, the limits of storage are not much more than one month at 37° C." (Bennett 1936.) Unfortunately this experiment did not confirm Bennett's findings. After only four days' storage the vaccine did not protect against 2 c.cms. virulent blood given 27 days later, both animals dying of rinderpest. Of the eight animals used, only two survived the injection of virulent blood—one in the six days group and one in the fifteen days group—but both had such severe reactions that it may be concluded that the vaccine conferred little, if any, immunity.

Bennett apparently does not state how he tested the immunity conferred by his vaccine. Possibly my test by injecting 2 c.cms. of virulent blood was far more severe than his, but it was the test used in all my experiments with goat adapted virus. It is possible, too, that my experimental animals were more susceptible than his and my virus more virulent than that used in his experiments.

(c) *Observations on the rise of temperature in thermos jars containing ice.*

The capacity of the thermos jars used is 1,500 c.cms. (roughly two and a half pints) and the price in Rangoon is three shillings and six pence each.

(i) A block of ice weighing 250 grams was put into each of six thermos jars. After 40 hours the temperature was 32° F. in No. 1 jar, after 64 hours it was 44° F. in No. 2 jar, and after 86 hours it was between 60° and 64° F. in the other four jars.

(ii) Various quantities of finely crushed ice were put into six thermos jars. Details of temperature rise and quantities of ice are as follows:—

Jar No.	Weight of Ice.	Temperature after 64 hours.	Temperature after 88 hours.
1	320 grams	33°F	—
2	330 grams	32°F	—
3	280 grams	—	64°F
4	355 grams	—	74°F
5	325 grams	—	59°F
6	330 grams	—	59°F

(iii) Twenty-four tubes of dried vaccine were put into each of three thermos jars, which were then filled with crushed ice (about 850 grams). At the end of 88 hours the temperatures recorded in the three jars were 59° F., 60° F., and 60° F.

(iv) Twelve thermos jars were filled with crushed ice (900 grams in each) and kept in an incubator at 97° F. The rise of temperature was as follows:—

JAR NO.	TEMPERATURE RECORDED.	
	After.	Temperature.
1 and 2	24 hours	33°F
3 and 4	48 hours	33°F
5 and 6	72 hours	33°F
7	96 hours	67°F
8	96 hours	69°F
9, 10, 11 and 12	120 hours	97°F

These small experiments show that as a refrigerant crushed ice is as effective as a block of ice; and that the temperature in a quart thermos jar filled with crushed ice remains at 32° F. for at least 72 hours, and does not reach that of the surrounding air for at least four days.

(*f*) *Observations on Dry Ice* (*Solid Carbon Dioxide*).

Dry ice was obtained from Madras, packed in insulated leather containers and carried in the ship's cold store. During the 84 hours in transit the weight of the dry ice decreased from 114 lb. to 45 lb. It was then stored at 42° F., and at this temperature the daily loss was 10 lb., so that there was a gradual increase in the percentage loss. On exposure to room temperature, which varied from 78° to 83° F., the loss was very rapid. One pound of dry ice was packed in each of six thermos jars, which were tightly corked. Within 24 hours all the ice in all the jars had evaporated. Blocks of dry ice, weighing twelve ounces, were put in aluminium tins with screwed-on lids. All the ice evaporated within four hours.

As a refrigerant for the despatch of dried vaccine in Burma dry ice is useless.

IV. APPLICATION OF EXPERIMENTAL RESULTS.

(*a*) *Preparation of dried goat spleen vaccine.*

For the preparation of this vaccine the goat is killed on the fourth day after injection with goat adapted rinderpest virus, the spleen removed aseptically, finely ground and rapidly dried. The material, which is not tested for sterility or potency, is despatched in a thermos jar of ice, emulsified with normal saline immediately before injection, and used in doses of one gram for 400 cattle and one gram for 2,400 buffaloes. The vaccine is used as a prophylactic measure both in clean areas and in natural outbreaks of the disease. The preparation of the vaccine will be described in detail.

Goats are obtained from Upper Burma, where the supply is apparently inexhaustible, and delivered at the Laboratory at a cost of ten shillings each. They are not specially selected, though big, healthy-looking animals are taken for preference. It is not possible, even if it were desirable, to obtain only males, and no matter at what time of the year animals are purchased kidding is of daily occurrence. Pregnant goats used for vaccine production do not abort, and if they did it would not reduce their value. The spleens of female goats are slightly bigger than those of males, and the percentage of loss in drying is the same. The average weight of the spleen of the last 59 female goats used was 55·5 grams and that of the last 24 male goats was 49·9 grams. The loss in weight in drying was 76·5 per cent. for spleens of female goats and 77·1 per cent. for spleens of male goats.

The strain of goat virus originated from Muktesar and was kindly supplied by the Director of Veterinary Services, Madras, in December, 1934. At Insein it has been kept going in goats either by direct blood inoculations on the day of bleeding, or by injection of blood or spleen pulp after storage at 42° F. for varying periods. In the earlier passages virus material from a single animal was on each occasion used, but latterly the pooled virus of the vaccine producers has been injected.

The reaction in goats has been dealt with elsewhere in this paper. The goats used here are so universally susceptible to rinderpest that it seems all may be used without reference to the temperature or other reaction. Of 615 infected for vaccine production, only 5 were discarded because of what appeared unsatisfactory temperature reactions.

The goat is bled to death and the spleen removed as quickly as possible through the left flank. The only treatment given to the skin is to clip the hair along the lines of incision and remove the loose hairs with a stiff brush. Using sterilized instruments the skin over the left flank is reflected and the abdominal contents allowed to drop down by an incision through the muscular coat in the mid-ventral line, the muscles in the flank being then incised without danger of puncturing the rumen. Lifting the ribs exposes the spleen, which is grasped with a pair of forceps and removed by snipping its attachments. A smear from each spleen is examined after staining for one minute by Giemsa, and the carcase is submitted to a cursory inspection. Of 610 spleens, ten were discarded because of fibrous peritonitis, and 14 because the goats had died of rinderpest before the 96th hour.

The spleens are minced in a Latapie pulper of 400 c.cms capacity. To preserve the cutting edges the knives are kept in 70 per cent. alcohol and washed in boiled water immediately before use the rest of the instrument being sterilized by boiling. The use of the Latapie pulper is a little wasteful in that some of the pulp is retained in the dead space between the piston and the grinding surface. Some pulp is also retained in the system of cutting edges, but to minimise the possibility of infection no attempt is made to salvage this material. The pulp is transferred to sterilized petri dishes through a short rubber tube attached to the outlet of the Latapie.

The pulp is dried in vacuo over calcium chloride, and for this purpose are used a Pfeiffer double stage vacuum pump, stout-walled desiccators, with an internal diameter of 9 inches and a depth of 8½ inches, and calcium chloride of good quality. Rapid drying of the pulp is attained only by attention to details. The bottom of the desiccator is covered with lumpy calcium chloride and a little white vaseline is rubbed on the opposing surfaces of the lid and rim of the desiccator after they have been carefully cleaned. The lid is well seated on to the desiccator so as to give an even distribution of the layer of vaseline. The tubulure and stopcock are also carefully cleaned and vaselined, and the pump connected up with the desiccator by means of pressure tubing. Care is taken to keep the pump filled with an adequate supply of clean oil. These details have been enumerated as on the few occasions when assistants reported failure to dry the pulp rapidly or completely, this was due to neglect of one or more of the points mentioned. A petri dish containing the pulp from about one spleen is put into a desiccator and the pump allowed to run for two or three minutes. The desiccator is placed in a refrigerator at 42° F., and after two hours the pulp is stirred to disturb the dry crust on the surface and to expose the moister material below. The air is again exhausted and the desiccator returned to the refrigerator. The following day the pulp is rapidly ground in a pestle and mortar, and transferred to drawn glass tubes, about 8 inches by $\frac{3}{4}$ inch, from which it is run into 2 inches by $\frac{1}{4}$ inch test tubes, which are closed with rubber corks and sealed by dipping in melted paraffin wax. This is done quickly so as not to heat the tube or its contents. The vaccine is stored in these tubes at 42° F. In emergencies the pulp is more rapidly dried by placing only small quantities in each desiccator and the vaccine issued within a few hours of the spleen being removed from the goat.

Vaccine is prepared weekly, the number of goats used being regulated by field requirements. This procedure was originally adopted as a matter of convenience but is now strictly adhered to because Haddow (1934) has shown that it is not possible to maintain the goat adapted virus for more than four generations if it is stored at 0° C. for more than ten days between each passage.

In the earlier experiments, to make the most of limited funds, use was made of whatever material was to hand. For this reason the tubes of vaccine were packed in the small aluminium tins used for photographic roll films. They were light and of a convenient size. As the tins of vaccine were packed in ice it was necessary to make them watertight, and for this purpose plasticine was used. With the adoption of the vaccine for routine inoculation in Burma it was possible to consider an improvement in the packing of the vaccine. More elaborate tins with screw caps and collars and rubber washers were devised and other methods of preventing the entrance of water were tried but the simple aluminium tin, with slip-on lid, which is made locally very cheaply, and the use of plasticine, remain the most satisfactory method of packing. A thermos jar, of two and a half pints capacity, is half filled with powdered ice, the tin of vaccine put on this ice, and the jar filled with powdered ice, which is rammed down firmly. The boxes containing the thermos jars receive rough handling in transport, and to guard against breakages powdered ice is used in preference to a single piece of ice. The cork of the thermos jar is sealed with a strip of plasticine, and the jar packed in a wooden box of such dimensions as to allow not less than four inches of sawdust, bran or paddy-husk between the jar and the box. The packing material acts as an insulator as well as a buffer against rough handling. To keep a check on thermos jars they are numbered and periodically tested.

It is usual for inoculators to arrive at their destination in the evening and commence work early the following morning. They are supplied with one hundred c.cms. and thirty c.cms. bottles, sodium chloride tablets, glass pestles and wedgewood mortars. Boiling cracks a glass mortar and melts the glue in the handle of a wedgewood pestle, but does not damage a glass pestle or a wedgewood mortar. Instruments and bottles are boiled the evening before inoculation, so that the following morning all instruments are sterilized but cold, there is no danger of killing the vaccine in a hot mortar, and there is an ample supply of cold sterile water for the preparation of normal saline. By the adoption of these simple measures the procedure has been made very nearly fool proof, which is a very necessary precaution.

Each tube contains about 0·25 gram vaccine, which is sufficient for one hundred cattle. The powder is emptied into the mortar, saline added and the powder rubbed up into a paste. More saline is added and the powder is completely emulsified. the whole procedure taking not more than 30 seconds. The emulsion is poured into a 100 c.cms. bottle, which is filled up with normal saline and thoroughly shaken. For buffaloes five c.cms. of this vaccine is put into a 30 c.cms. bottle, which is then filled up with normal saline giving thirty doses of one c.cm. each. The vaccine is injected subcutaneously, after washing the skin with soap and water. The hair is not clipped and inoculators are advised not to use any disinfectant, though it is feared that many still cling to the old belief of the absolute necessity of scrubbing the site of injection with tincture of iodine or with one of the coal tar disinfectants. Inoculators are allowed to use the spleen powder up to thirty days after despatch from the Laboratory, provided the ice in the thermos jar is replenished every three days. They are not permitted to use it later than six days after ice was last added and vaccine not used on the day emulsified with normal saline must be discarded.

(*b*) *The use of the vaccine in the field.*

When first used in an area free from rinderpest for 30 years, 0·1 gram of the vaccine caused a mortality of about one per cent. in cattle and of about 20 per cent. in one lot of buffaloes. It was presumed that this heavy mortality was due to long freedom from the disease and the consequent lack of natural resistance. It was then thought that the dosage could with advantage be varied with the years of freedom from the disease, and for this reason all requisitions for vaccine were accompanied by a history of the disease in the area where the cattle were to be inoculated. Dosage was graded accordingly and used as follows:—

Years area free from Rinderpest.	*Doses of vaccine for cattle.*
Up to 2 years	200 doses per gram.
2 to 5 years	400 doses per gram.
6 to 9 years	600 doses per gram.
10 or longer	1,000 doses per gram.

A fairly extensive trial showed that the dose did not materially influence the reaction in cattle but that many buffaloes were killed by a dose which was safe for cattle. The dose was, therefore(fixed at one gram of powder for 400 cattle, and one gram for 2,400 buffaloes. Experiments have shown that one-tenth of the dose fixed for cattle is just as effective, but the main reason for fixing the dose at one gram for 400 cattle is that very rarely, if ever, is this number inoculated in one day. Each villager owns only a few animals, so that even if it were possible for inoculators to do more than 400 in one day, this number would not be available. Inoculators have instructions to use vaccine on the day it is emulsified, which in practice means that it is used within six hours. The powder is not weighed but the amount is gauged fairly accurately by comparison with a weighed quantity in a similar tube. It would, however, be more difficult to gauge a smaller amount, so that all things considered it is deemed advisable to waste a little vaccine rather than reduce the dose to the limits indicated possible by experiments. The vaccine is now being used wherever it is possible to deliver it in a viable form, which excludes only a very few areas in Burma. It is used in infected herds during an outbreak, in herds where outbreaks are apt to occur, and to establish a zone of immunized cattle around an infected area. This, however, is outside my sphere of action, and will not be further commented upon. Details of the numbers of cattle and buffaloes inoculated with the vaccine are given in the table immediately below. The mortality said to have been caused by the vaccine is also shown. All these inoculations were carried out by officers of the

Month.	Cattle.		Buffaloes.	
	Number inoculated.	Deaths up to 30 days after inoculation.	Number inoculated.	Deaths up to 30 days after inoculation.
1935—October	827	3	47	1
November	1,144	2	147	1
December	702	3	251	45
1936—January	986	1	—	—
February	3,075	21	—	—
March	2,698	18	1,012	24
April	4,325	46	733	11
May	13,046	9	638	25
June	25,923	82	550	28
July	27,259	94	3,457	107
August	22,110	36	2,054	93
September	13,606	33	2,832	66
October	22,566	28	3,399	109
November	19,688	19	3,138	84
December	25,055	17	2,116	41
1937—January	28,429	16	1,214	12
February	38,720	32	3,340	61
March	52,872	53	4,768	98
April	30,046	41	3,035	79
May	43,166	65	2,257	149
June	27,628	24	1,158	15
July	24,487	14	1,036	16
August	15,501	23	985	9
September	27,712	24	1,946	14
October	46,392	88	4,013	106
November	41,183	57	3,192	44
December	37,502	31	3,502	58
1938—January	46,025	20	3,476	48
February	57,536	24	3,393	24
March	59,583	20	4,785	17
TOTAL	759,792	944	62,474	1,385

Veterinary Department, many of whom are of limited education. Since in all parts of the country big numbers of cattle have been inoculated without any mortality it is reasonable to conclude that in nearly every case death after inoculation was not due to the vaccine, but to other diseases, or to poverty and neglect. Enquiry has shown that in some cases poverty-stricken animals, especially buffaloes, were turned out into the jungle after inoculation and neglected, many dying of starvation. Animals dying in infected herds were reported as having been killed by the vaccine, whereas many probably died of natural infection. Nevertheless the figures in the table include all deaths up to about 30 days after inoculation, and show a total mortality of 944 out of 759,792 cattle, and 1,385 out of 62,474 buffaloes. During the fifteen months ending 31st March, 1938, which excludes the heavy mortality when the vaccine was first introduced, of 576,782 cattle inoculated, 532 died, and of 42,100 buffaloes, 750 died. As the table is based on reports received from inoculators up to the 30th April, 1938, the figures are necessarily incomplete.

(c) *The advantages of the vaccine.*

The vaccine may reasonably be described as cheap, safe, and effective.

As at present used in Burma the vaccine is many times more expensive than it would be if used in the doses shown to be effective, but even so it is estimated that the cost, including goats, apparatus, packing and freight amounts to about four shillings a thousand doses. The preparation of a vaccine from cattle would be a hazardous undertaking in Burma, where the supply of susceptible cattle is limited to far away areas not free from natural outbreaks of the disease, so that at any moment one's supply might be cut off. Goats, on the other hand, are obtainable everywhere, and all seem equally susceptible to the disease. They are, moreover, cheap and the cost of their upkeep is small. The breakages of thermos jars, which is perhaps the main item in the cost of bottling and despatch, is surprisingly small, and has amounted to only 12 out of 1,507 sent out. There is a great saving in time and material in tubing the vaccine, as 100 doses can be put into a small agglutination tube in a few seconds, while the cost of transport on a box of vaccine from the Laboratory to the inoculators' headquarters averages about one shilling.

A gross mortality of less than one in a thousand cattle during the fifteen months ending 31st March, 1938, shows that the vaccine is safe. Repeated attempts at the Laboratory to transmit the disease to Kyaukpyu cattle by contact have failed, and field observations support these findings. In each of the Laboratory experiments ten Kyaukpyu cattle were mixed in a small loose box and five given goat virus. All the cattle were fed and watered from the same utensils, and although the inoculated cattle reacted to the virus and were kept in contact with the test cattle for 30 days these showed no rise in temperature and later reacted typically to the subcutaneous injection of goat virus.

With buffaloes the position is a little uncertain. Edwards (1927b) stated that in India the buffalo is not regarded as particularly susceptible. In the Philippines buffaloes are considered much more susceptible than cattle. In Burma the buffalo is extremely susceptible and in any outbreak of rinderpest a mortality of 100 per cent. is expected, leaving no recovered animals and consequently no animals with any acquired immunity. In the Philippines it was found necessary to give buffaloes three injections of chloroform vaccine, as the immunity developed from one injection was insufficient to protect them against exposure to natural infection. In Burma inoculators have reported that dried goat spleen in a dose of 1 gram for 2,400 buffaloes has at times caused severe reactions and a few deaths. Spread of the disease to uninoculated buffaloes by contact with inoculated buffaloes has also been reported. It is hoped that at Insein facilities will soon be available for work on buffaloes and that then the immunization of these animals will be further investigated.

The vaccine transmits no blood parasites and causes no local reaction. As only the spleen is used and this can be quickly and aseptically removed, there is little danger of contamination. It would be otherwise if tonsils and lymph glands were incorporated in the vaccine. Field work is sometimes performed under great difficulties, and it is conceivable that contamination might occur during emulsification of the vaccine in the mortar. On two occasions at the Laboratory vaccine was emulsified in a small cattle enclosure while many assistants intentionally created as much dust as possible, but even then the vaccine produced no local reaction. Emulsification may also be effected by prolonged shaking of the spleen powder in a bottle of normal saline. This is a longer and more tiring process which does not satisfactorily break up the spleen tissue, and Haddow (1934) has shown that watery extracts are much less virulent than tissues. The use of the mortar has no disadvantages and with it complete emulsification is effected in 30 seconds.

Experiments have shown that the vaccine confers immunity which is serviceable in 24 hours and solid in 48 hours, and endures for at least two years. As immunity is not lasting and as that conferred by vaccines can be reinforced by virus, it is reasonable to presume that loss of immunity is gradual and not sudden, and can be reinforced by exposure to natural outbreaks of the disease. In estimating the duration of immunity, animals which might have been exposed to natural infection should, therefore, be disregarded, and failure to do this has probably contributed to the disagreement on the subject of immunity conferred by tissue vaccines and by serum-virus. In my own work great care was taken to exclude any possibility of immunity being reinforced and experiments have shown that the immunity lasts for at least twenty-five months. Reports from the field confirm these Laboratory findings.

Since the vaccine is kept on ice its effectiveness is assured and one would expect field results to be in accordance with Laboratory experiments. In many countries ice would present an insurmountable difficulty but in Burma it is no handicap. It is available on trains, steamers and ferry boats. It is made in most of the smaller towns and procurable in many out of the way villages, the price per pound varying from half a penny in the larger towns to two pence in the villages, though in a few distant villages it goes up to as much as sixpence. Figures 7, 8 and 9 give a good idea of the temperatures to which vaccine would be exposed if not packed on ice.

V. DISCUSSION.

The term " strains " in connection with rinderpest virus has been used as a matter of convenience, and should not be taken to indicate the existence of a plurality of strains. The difference is one of virulence or degree. I have on several occasions failed to maintain by passage in susceptible cattle strains obtained from natural outbreaks of the disesae. One strain of cattle virus was much less virulent for goats than the goat adapted virus which never kills our most susceptible cattle.

The cattle of Burma vary greatly in their susceptibility to rinderpest. Mention has also been made of this in other countries, and it is possible that this variation in susceptibility and the difference in virulence of strains account for much of the lack of uniformity reported in immunization against rinderpest. This is possibly the reason for my failure to confirm the results obtained by Bennett with his glycerine vaccine.

Although doubt has been cast on the living nature of at least some of the filterable viruses, there is some justification for speaking of " attenuation " and " killed " in connection with rinderpest virus. At any rate it is convenient to do so.

Curasson (1930) has condemned as useless vaccines prepared from attenuated virus. Although killed virus will immunize against such infections as herpes, psittacosis and distemper, it is open to doubt whether any really effective rinderpest vaccine does not contain living virus, however attenuated. In 1928 Daubney stated " Whether the reaction to inoculation with vaccines prepared in weak formalin depends upon the presence of a minimal dose of unchanged living virus, the size of the dose being the determining factor in the severity of the reaction, if any, provoked; or whether the virus is in any way progressively attenuated by the action of formalin without being actually killed, are problems that must await solution by further experiment ". Edwards (1925) made a most interesting observation. He stored blood virus at body heat under paraffin. After three months the virus had become inert for cattle, but intense passage through rabbits brought back its original virulence for cattle. In view of the fact that Walker (1929) showed that in blood, virus becomes inert in a matter of hours, and that Edwards was unable to preserve the potency of blood virus for more than eight days, it would seem that when apparently dead, rinderpest virus may be only greatly attenuated. On the other hand Shilston (1917) noted that at Muktesar defibrinated blood remained virulent for 40 days at room temperature.

The most lasting immunity is that conferred by virulent cattle virus in combination with either immune serum or vaccine. It has been shown that the immunity conferred by goat virus and serum is not as lasting as that conferred by cattle virus and serum. Investigators have searched for a vaccine to overcome the disadvantages of the serum-virus method, but while they have gained in cost and safety they have lost in duration of immunity. All vaccines prepared from cattle tissues are rendered safe by storage or by exposure to chemicals. Even when they have reached the safety stage they may still kill highly susceptible cattle, as was shown by Cooper (1931), indicating that they still contain active virus. In connection with tissue vaccines, " safety " is a purely relative term. After storage at 42° F. for five months even the most virulent cattle virus loses its power to kill susceptible cattle, and instead immunizes them. An infinitesimal quantity of fully potent virulent virus kills a susceptible animal but the more attenuated that virus becomes the greater the quantity required to produce a reaction. Some consider that rinderpest virus does not become attenuated and that the effect of such material is due to the survival in the injected material of minute quantities of virus, but considering the very minute quantities of virulent material required to kill a susceptible animal and the regularity with which reactions may be provoked by large quantities of " attenuated " virus, a more reasonable explanation is that all or nearly all the virus has become attenuated, and that none, or very little, has been killed. This subject is of more than academic interest as if it can be shown experimentally that tissue vaccines depend upon living but attenuated virus for their immunizing effect, the necessity for not allowing attenuation to go too far is obvious. There is no doubt that for goat adapted virus to immunize it must be fully potent. 0·01 gram of dried spleen is an unreliable immunizing agent after exposure for six days at 97° F. But even after seven days at this temperature 0·5 gram will immunize, indicating that a large quantity of attenuated virus may be successfully substituted for a small quantity of fully potent virus. Of great practical importance is the short time for which goat virus remains effective at a temperature of 97° F. A small preliminary experiment indicated that by exposure for twelve hours to 80° F. and 97° F. alternately the virus becomes attenuated as rapidly as it does on exposure to a constant temperature of 97° F. It is not known if the virus may be attenuated to a required degree by exposure to a temperature of 97° F. and then maintained at this strength by storage at about zero.

In Burma during the hot months of March, April and May vaccine despatched to the field is exposed to temperatures of 97° F. and more for many hours each day and in many parts during these months the temperature never drops below 85° F. After 6 days vaccine stored alternatively for twelve hours at 97° F. and for twelve hours at 80° F. failed to immunize in doses of 0·01 gram so that vaccine despatched from the Laboratory not packed on ice would be exposed to fairly high temperatures, and rapidly lose its immunizing properties. Only with vaccine kept on ice can one hope to reproduce Laboratory results. Fortunately in Burma ice is easily obtainable and for that reason keeping the vaccine on ice presents no difficulty. The only alternative to this method of despatch is for inoculators to prepare vaccine as required. Very few of our inoculators could be entrusted with this and the procedure would in any case meet with opposition as it would offend against the religious principles of the people.

VI. SUMMARY.

(*a*) The various methods of immunizing against rinderpest are briefly summarised, the use of goat adapted virus being dealt with more fully.

(*b*) Experiments leading up to the production of a vaccine prepared from dried goat spleen are described.

(*c*) The dried vaccine is prepared from the spleen of a goat destroyed on the fourth day after injection with goat adapted virus. The spleen is minced in a Latapie and the pulp rapidly dried in vacuo over calcium chloride.

(*d*) Packed in a thermos jar of ice, which is replenished every third day, the vaccine is emulsified in normal saline immediately before use, and used in a dose of one gram for 400 cattle and one gram for 2,400 buffaloes, although experiments have shown that 0·00025 gram is equally effective for cattle.

(e) Immunity commences within twenty-four hours and is solid within forty-eight hours. It endures for at least 755 days, the limit to which it has so far been tested.

(*f*) 576,782 cattle and 42,100 buffaloes were inoculated during the fifteen months ending 31st March, 1938. The mortality in them was 0·092 per cent. in cattle and 1·78 per cent. in buffaloes. Complete reports are not yet available, but those received show that from October, 1935, to 31st March, 1938, 759,792 cattle and 62,474 buffaloes were inoculated.

(*g*) The opinion is expressed that to confer lasting immunity tissue vaccines must contain living virus.

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APPENDIX.

Tables 1 to 18.—Giving details of experiments described in the text.

NOTE.—1. In all tables (K) indicates a highly susceptible Kyaukpyu animal, and (L) indicates an animal bred in Burma, of unknown susceptibility, but usually more resistant to rinderpest than the Kyaukpyu cattle.

2. In tables 1 to 18 the temperature in all cases refers only to that recorded before eight o'clock in the morning, the evening temperature being probably accentuated by the heat of the day and for that reason being disregarded.

3. In immunity tests "Immune" indicates no reaction whatever.

TABLE 1. (EXPERIMENT 1.)

The passage of the Kungyangon strain of rinderpest cattle virus in Kyaukpyu cattle.

Passage Number.	ANIMAL.			INJECTION OF VIRUS.			REACTION.				FATE.	
	Number.	Age in years.	Sex.	Date Injected.	Material used.		Day of initial rise.	Day Peak reached	Peak tempera-ture.	Rise in tempera-ture.	Day died.	Day destroy-ed.
					Blood.	Dried spleen powder.						
1	320	4	Cow. .	2/ 4/35	Blood from ox and buffalo with naturally acquired Rinderpest	—	3rd	8th	103·6°F.	3·4°F.	11th	—
2	278	1½	Cow...	11/ 4/35	Ex. 320 direct..	—	3rd	5th	104·2°F.	4°F.	—	8th
3	289	1½	Bull...	23/ 4/35	—	Ex. 278 stored for 4 days at 42°F.	3rd	5th	104°F.	4°F.	9th	—
4	298	2½	Bull...	28/ 4/35	Ex. 289 direct.	—	3rd	5th	104°F.	4°F.	9th	—
5	291	1	Bull...	3/ 5/35	Ex. 298 direct.	—	3rd	5th	105·4°F.	5·2°F.	—	5th
6	314	3	Cow. .	8/ 5/35	Ex. 291 direct.	—	2nd	6th	105·8°F.	5·4°F.	9th	—
7	309	2	Cow..	13/ 5/35	Ex. 314 direct.	—	2nd	5th	106°F.	5·8°F.	—	6th
8	274	1½	Cow...	18/ 5/35	Ex. 309 direct.	—	3rd	5th	105·2°F.	5°F.	—	6th
9	283	1	Bull...	23/ 5/35	Ex. 274 direct.	—	3rd	5th	102·6°F.	3°F.	10th	—
10	300	1	Bull...	28/ 5/35	Ex. 274 and 283 direct	—	3rd	4th	104·2°F.	4°F.	—	6th
11	311	1½	Cow...	2/ 6/35	Ex. 300 direct.	—	2nd	5th	104°F.	3·6°F.	8th	—
12	306	2	Cow. .	12/ 6/35	Ex. 311 direct.	—	3rd	5th	104·6°F.	4·6°F.	—	2nd
13	360	3½	Bull..	22/ 7/35	—	Ex. 306 stored for 39 days at 42°F.	2nd	6th	106°F.	6°F.	9th	—
14	348	3½	Bull...	27/ 7/35	Ex. 360 direct.	—	3rd	4th	103·8°F.	3·8°F.	7th	—
15	380	3	Bull...	1/ 8/35	Ex. 348 direct.	—	3rd	6th	105·4°F.	5·8°F.	9th	—
16	353	1	Cow...	11/ 8/35	Ex. 380 direct.	—	3rd	5th	105·6°F.	5·2°F.	—	5th
17	391	1	Bull...	16/ 8/35	Ex. 353 direct.	—	3rd	3rd	105°F.	5°F.	—	5th

TABLE 1—(*cotntinued*).

Passage Number.	Animal.			Injection of Virus.			Reaction.				Fate.	
	Number.	Age in years.	Sex.	Date Injected.	Material used.		Day of initial rise.	Day Peak reached.	Peak tempera-ture.	Rise in tempera-ture.	Day died.	Day destroy-ed.
					Blood.	Dried spleen powder.						
18	352	2	Bull...	30/ 8/35	—	Spleen pulp ex. 391 in 50 per cent. Glycerine Saline stored for 9 days at 42°F.	3rd	4th	105°F.	4·6°F.	—	6th
19	374	2	Cow...	4/ 9/35	Ex. 352 direct.	—	3rd	6th	104·6°F.	3·4°F.	10th	—
20	383	1½	Bull...	9/ 9/35	Ex. 374 direct.	—	3rd	6th	105°F.	5°F	9th	—
21	371	4	Cow...	19/ 9/35	Ex. 383 direct.	—	3rd	5th	105·4°F.	4·8°F.	—	5th
22	351	1½	Cow...	1/11/35	—	Ex. 371 stored for 37 days at 42°F.	4th	4th	104·6°F.	3·6°F.	—	5th
23	390	3½	Cow...	17/11/35	—	Ex. 351 stored for 12 days at 42°F.	2nd	4th	105·4°F.	4·8°F.	8th	—
24	392	5	Cow...	22/11/35	Ex. 390 direct.	—	3rd	4th	103·4°F.	3·4°F.	9th	—
25	463	5	Cow...	27/11/35	Ex. 392 direct.	—	3rd	4th	103·4°F.	2·4°F	—	5th
26	474	1	Bull...	28/12/35	—	Ex. 463 stored for 29 days at 42°F.	2nd	5th	103·4°F.	4·4°F.	—	5th
27	456	4	Cow...	16/ 1/36	—	Ex. 474 stored for 17 days at 42°F.	3rd	5th	102·6°F.	3·6°F.	11th	—
28	464	2½	Cow...	21/ 1/36	Ex. 456 direct.	—	3rd	4th	103°F.	3°F.	10th	—
29	450	3	Cow...	26/ 1/36	Ex. 464 direct.	—	3rd	6th	104·8°F.	4·8°F.	—	6th
30	470	2	Cow...	15/ 2/36	—	Ex. 450 stored for 16 days at 42°F.	3rd	5th	104°F.	5°F.	—	5th
31	446	1	Cow...	24/ 2/36	—	Ex. 470 stored for 5 days at 42°F.	2nd	6th	105°F.	5·6°F.	—	6th
32	471	2	Bull...	15/ 3/36	—	Ex. 446 stored for 16 days at 42°F.	3rd	5th	103·4°F.	3·8°F.	—	5th
33	457	2½	Cow...	3/ 4/36	—	Ex. 471 stored for 15 days at 42°F.	3rd	4th	104·8°F.	3·8°F.	—	5th
34	475	2½	Cow...	22/ 4/36	—	Ex. 457 stored for 15 days at 42°F.	3rd	5th	104°F.	3·8°F.	—	5th
35	435	3½	Bull...	11/ 5/36	—	Ex. 475 stored for 15 days at 42°F.	3rd	5th	104·8°F.	3·6°F.	—	5th
36	443	5	Bull...	30/ 5/36	—	Ex. 435 stored for 15 days at 42°F.	3rd	5th	105·6°F.	4·6°F.	—	5th
37	499	3	Cow...	11/ 6/36	—	Ex. 443 stored for 15 days at 42°F.	3rd	4th	104·2°F.	3·2°F.	8th	—
38	514	1	Cow...	16/ 6/36	Ex. 499 direct.	—	3rd	4th	104·6°F.	3·6°F.	8th	—
39	516	2	Cow...	21/ 6/36	Ex. 514 direct.	—	3rd	3rd	103·8°F.	3·2°F	—	5th

TABLE 1—(*continued*).

Passage Number.	ANIMAL.			INJECTION OF VIRUS.			REACTION.				FATE.	
	Number.	Age in years.	Sex.	Date Injected.	Material used.		Day of initial rise.	Day Peak reached.	Peak temperature.	Rise in temperature.	Day died.	Day destroyed.
					Blood.	Dried spleen powder.						
40	522	1½	Cow...	1/ 7/36	—	Ex. 516 stored for 6 days at 42°F.	3rd	5th	104·8°F.	4·6°F.	—	5th
41	525	2	Cow...	1/ 8/36	—	Ex. 522 stored for 17 days at 42°F.	4th	8th	105°F.	5°F.	—	Recovered.
42	531	1½	Cow...	6/ 8/36	Ex. 525 direct.	—	3rd	5th	105·2°F	5·2°F	—	5th
43	498	3	Cow...	2/ 9/36	—	Ex. 531 stored for 23 days at 42°F.	2nd	5th	104°F.	3·6°F.	—	5th
44	528	4	Cow...	24/ 9/36	—	Ex. 498 stored for 18 days at 42°F.	4th	5th	104·8°F	4·4°F.	—	5th
45	527	3½	Cow...	17/10/36	—	Ex. 528 stored for 19 days at 42°F.	4th	5th	104·4°F.	3·6°F.	—	5th
46	502	2	Cow...	18/11/36	—	Ex. 527 stored for 28 days at 42°F.	3rd	5th	105·6°F.	5·2°F.	—	5th
47	492	3	Bull...	12/12/36	—	Ex. 502 stored for 20 days at 42°F.	3rd	5th	104°F.	4°F.	—	5th
48	486	3	Cow...	16/ 1/37	—	Ex. 492 stored for 31 days at 42°F.	3rd	5th	103·2°F	4·2°F.	—	5th
49	532	2	Cow...	20/ 2/37	—	Ex. 486 stored for 31 days at 42°F.	3rd	5th	104°F.	3·6°F.	—	5th
50	534	5½ months	Heifer	19/ 3/37	—	Ex. 532 stored for 23 days at 42°F.	2nd	5th	104·6°F.	3·6°F.	—	5th
51	491	4	Cow...	17/ 4/37	—	Ex. 534 stored for 25 days at 42°F.	3rd	5th	104·6°F.	4·6°F.	—	5th
52	545	3½	Bull...	15/ 5/37	—	Ex. 491 stored for 24 days at 42°F.	2nd	5th	104·2°F.	4·4°F.	—	5th
53	550	2½	Cow...	21/ 6/37	—	Ex. 545 stored for 33 days at 42°F.	2nd	4th	104·2°F.	4·2°F.	—	5th
54	543	2	Bull...	21/ 7/37	—	Ex. 550 stored for 26 days at 42°F.	2nd	4th	104°F.	4°F.	—	5th
55	542	2	Bull...	23/ 8/37	—	Ex. 543 stored for 29 days at 42°F.	2nd	3rd	104°F.	4°F.	—	5th
56	555	3	Cow...	22/ 9/37	—	Ex. 542 stored for 26 days at 42°F.	2nd	5th	104·6°F.	4·6°F.	—	5th
57	546	3½	Bull...	25/10/37	—	Ex. 555 stored for 29 days at 42°F.	3rd	5th	105°F.	5°F.	—	5th
58	551	2	Cow...	27/11/37	—	Ex. 546 stored for 29 days at 42°F.	2nd	5th	104·8°F.	4·8°F.	—	5th
59	564	2½	Cow...	27/12/37	--	Ex. 551 stored for 26 days at 42°F.	2nd	5th	104°F.	4·2°F.	—	5th

TABLE 2. (EXPERIMENT 2.)

To determine the effect of storage at 42° F. on Rinderpest Virus (Cattle Strain) in the form of powdered spleen dried in Vacuo over Calcium Chloride.

Cattle Number.	INJECTION OF VIRUS.			REACTION.					IMMUNITY TEST.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11	12
		Gram.	Days.								
366 (K)	5/8/35	0·01	77	5th	8th	105·6°F.	4·8°F.	Died on 11th day.	—	—	—
381 (K)	5/8/35	0·05	77	5th	8th	105°F.	3·8°F.	Died on 12th day.	—	—	—
358 (K)	27/7/35	0·01	100	9th	13th	104·4°F.	3·8°F.	Died on 16th day.	—	—	—
354 (K)	27/7/35	0·01	100	10th	12th	104·2°F.	2·2°F.	Died on 15th day.	—	—	—
385 (K)	28/9/35	0·01	133	4th	5th	103·8°F.	2·4°F.	Died on 11th day.	—	—	—
377 (K)	28/9/35	0·01	133	7th	9th	105·4°F.	4·2°F.	Recovered	2/12/35	2 c.cm.	Immune.
321 (K)	6/4/35	0·8	177	5th	8th	105°F.	4·4°F.	Recovered........	28/ 4/35	5 c.cm.	Immune.
304 (K)	6/4/35	1·6	177	7th	8th	103·2°F.	1·2°F.	Recovered........	28/ 4/35	5 c.cm.	Immune.
301 (K)	6/4/35	1·6	177	4th	8th	105°F.	4·2°F.	Recovered	28/ 4/35	5 c.cm.	Immune.
298 (K)	Control to	Nos. 321,	304 and 301	—	—	—	—	—	28/ 4/35	5 c.cm.	Died on 9th day.

NOTE.

The Virus used on Nos. 366, 381, 385 and 377 was derived from Cattle Bovine No. 309 (Kungyangon Strain).

The Virus used on Nos. 358 and 354 was derived from Cattle Bovine No. 309 (Kungyangon Strain).

The Virus used on Nos. 321, 304 and 301 was derived from Buffalo No. 33 (Moulmein Cattle Strain).

TABLE 3. (EXPERIMENT 3.)

To determine the viability at 42° F. of Goat Adapted Rinderpest Virus in the form of a 20 per cent. emulsion of spleen pulp in 50 per cent. Glycerine-Saline.

Goat Number.	INJECTION OF VIRUS.			REACTION.				
	Date Injected.	Quantity of 20 Per cent. Spleen Emulsion.	Period Stored at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
255..........	17/12/34	2 c.cm.	20 days	2nd	3rd	105°F.	3·6°F.	Recovered.
273..........	6/ 1/35	2 c.cm.	40 days	3rd	4th	105°F.	3°F.	Recovered.
281..........	17/ 1/35	3 c.cm.	51 days	2nd	3rd	104°F.	2·2°F.	Died on 6th day.
289..........	25/ 1/35	2 c.cm.	60 days	2nd	5th	105·8°F.	4·2°F.	Recovered.
288..........	16/ 2/35	2 c.cm.	80 days	3rd	4th	102·8°F.	1·4°F.	Recovered.
306..........	14/ 3/35	2 c.cm.	102 days	8th	9th	101·4°F.	1·4°F.	Recovered.
353..........	2/ 4/35	2 c.cm.	121 days	16th	17th	104·8°F.	2·8°F.	Recovered.
332..........	22/ 4/35	2 c.cm.	141 days	3rd	8th	104·8°F.	2·8°F.	Recovered.
313..........	13/ 5/35	2 c.cm.	162 days	No distinct thermal reaction.				Died on 35th day
364..........	24/ 5/35	2 c.cm.	173 days	6th	10th	104·6°F.	2·6°F.	Died on 26th day.
370..........	14/ 6/35	2 c.cm.	193 days	5th	7th	105°F.	2·6°F.	Recovered.
375..........	2/ 7/35	2 c.cm.	211 days	10th	11th	104·6°F.	2·2°F.	Died on 24th day.
380..........	22/ 7/35	2 c.cm.	231 days	8th	9th	104·4°F.	1·2°F.	Recovered.

TABLE 4. (EXPERIMENT 4.)

To determine the viability at 83° to 86° F. of Goat Adapted Rinderpest Virus in the form of a 1 per cent. emulsion spleen pulp in 50 per cent. Glycerine-Saline.

Goat Number.	Injection of Virus.			Reaction.				
	Date Injected.	Quantity of 1 Per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Period Stored at 83° to 86°F. after two days at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
322..........	26/3/35	2 c.cm.	3 days	3rd	11th	104°F.	2·6°F.	Recovered.
323..........	26/3/35	2 c.cm.	3 days	2nd	14th	104·8°F.	4·2°F.	Recovered.
324..........	26/3/35	2 c.cm.	3 days	4th	9th	102·8°F.	2·4°F.	Recovered.
312..........	29/3/35	2 c.cm.	6 days	3rd	19th	104·4°F.	3·6°F.	Recovered.
329..........	29/3/35	2 c.cm.	6 days	2nd	13th	104·6°F.	2·2°F.	Recovered.
336..........	29/3/35	2 c.cm.	6 days	1st	3rd	102·4°F.	1·8°F.	Recovered.
328..........	1/4/35	2 c.cm.	9 days	4th	9th	104·6°F.	2·4°F.	Recovered.
346..........	1/4/35	2 c.cm.	9 days	9th	11th	103·2°F.	1·2°F.	Recovered.
340..........	1/4/35	2 c.cm.	9 days	3rd	16th	105·2°F.	3·2°F.	Recovered.

TABLE 5. (EXPERIMENT 5.)

To determine the immunizing properties of varying doses of Rinderpest Goat Spleen Vaccine in the form of a 4 per cent. emulsion of spleen pulp in 50 per cent. Glycerine-Saline after storage for four days at 42° F. followed by two days at 80° F.

Cattle Number.	INJECTION OF VACCINE.		REACTION.					IMMUNITY TEST.		
	Date Injected.	Quantity of 4 per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11
245 (L)...	23/2/35	1 c.cm.	12th	13th	101·4°F.	1·6°F.	Recovered.	28/4/35	2 c.cm.	Rise in temperature from 100°F to 103·8°F. Diarrhoea Mouth lesions. Recovered.
242 (L)..	23/2/35	1 c.cm.	9th	13th	101·4°F.	1·4°F.	Recovered.	8/5/35	2 c.cm.	Rise in temperature from 100°F to 104·6°F. Diarrhoea. Severe mouth lesions. Recovered.
249 (L)..	23/2/35	1 c.cm.	12th	14th	102·8°F.	1·6°F.	Recovered.	8/5/35	2 c.cm.	Rise in temperature from 100°F to 102·4°F. Recovered.
254 (L)...	23/2/35	2 c.cm.	10th	11th	101·4°F.	1·2°F.	Recovered.	28/4/35	2 c.cm.	Rise in temperature from 100°F to 103·6°F. Diarrhoea. Mouth lessions. Recovered.
261 (L)...	23/2/35	2 c.cm.	6th	13th	101·2°F.	1·0°F.	Recovered.	8/5/35	2 c.cm.	Died of Rinderpest on 19th day.
271 (L)...	23/2/35	2 c.cm.	No	Reaction				8/5/35	2 c.cm.	Died of Rinderpest on 13th day.
246 (L)...	23/2/35	3 c.cm.	12th	13th	101·8°F.	1·8°F.	Recovered.	28/4/35	2 c.cm.	Rise in temperature from 100°F to 104·0°F. Diarrhoea. Severe mouth lesions. Recovered.
262 (L)...	23/2/35	3 c.cm.	No	Reaction				8/5/35	2 c.cm.	Rise in temperature from 101°F to 103·6°F. Diarrhoea. Severe mouth lesions. Recovered.
243 (L)...	23/2/35	3 c.cm.	12th	13th	102·0°F.	1·0°F.	Recovered.	8/5/35	2 c.cm.	Immune.
260 (L)...	23/2/35	4 c.cm.	10th	11th	101·6°F.	0·8°F.	Recovered.	28/4/35	2 c.cm.	Immune.
256 (L)...	23/5/35	4 c.cm.	No	Reaction				8/5/35	2 c.cm.	Rise in temperature from 100°F to 104·6°F. Diarrhoea. Severe mouth lesions. Reco-

TABLE 6. (EXPERIMENT 6.)

To determine the duration of immunity conferred by Rinderpest Goat Spleen Vaccine in the form of a 1 per cent. emulsion of spleen pulp in 50 per cent. Glycerine Saline after storage for—

(1) 10 days at 42° F.
(2) 2 days at 42° F. followed by 2 days at 84° to 86° F.
(3) 2 days at 42° followed by 8 days at 84° to 86° F.

	INJECTION OF VACCINE.			REACTION.					IMMUNITY TEST.		
Cattle Number.	Date Injected.	Quantity of 1 per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
240 (L)...	31/ 3/35	2 c.cm.	10 days at 42°F.	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
248 (L)...	31/ 3/35	2 c.cm.	„	3rd	4th	105·6°F.	4·6°F.	Recovered	18/ 5/35	2 c.cm.	Immune.
267 (L)...	31/ 3/35	2 c.cm.	„	No Reaction.					18/ 5/35	2 c.cm.	Immune.
275 (K)...	31/ 3/35	2 c.cm.	„	No Reaction.					18/ 5/35	2 c.cm.	Immune.
282 (K)...	31/ 3/35	2 c.cm.	„	6th	6th	103·2°F.	2·2°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
302 (K)...	31/ 3/35	2 c.cm.	„	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
263 (L)...	25/ 3/35	2 c.cm.	2 days at 42°F. followed by 2 days at 84° to 86°F.	2nd	4th	102·8°F.	2·4°F.	Recovered.	8/ 5/35	2 c.cm.	Severe temperature reaction with Diarrhoea and mouth lesions. Recovered.
247 (L)...	25/ 3/35	2 c.cm.	„	No Reaction.					8/ 5/35	2 c.cm.	„
255 (L)...	25/ 3/35	2 c.cm.	„	No Reaction.					18/ 5/35	2 c.cm.	„
264 (L)...	25/ 3/35	2 c.cm.	„	3rd	5th	101·8°F.	1·8°F.	Recovered.	18/ 5/35	2 c.cm.	„
288 (K)...	25/ 3/35	2 c.cm.	„	No Reaction.					8/ 5/35	2 c.cm.	„
319 (K)...	25/ 3/35	2 c.cm.	„	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 10th day.
299 (K)...	25/ 3/35	2 c.cm.	„	3rd	6th	100·8°F.	1·8°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
303 (K)...	25/ 3/35	2 c.cm.	„	No Reaction.					18/5/ 35	2 c.cm.	Died of Rinderpest on 9th day.

TABLE 6—(*continued*).

Cattle Number.	Injection of Vaccine.			Reaction.					Immunity Test.		
	Date Injected.	Quantity of 1 per cent. Emulsion of Spleen Pulp in 50 per cent. Glycerine-Saline.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
253 (L)...	31/ 3/35	2 c.cm.	2 days at 42°F. followed by 8 days at 84° to 86°F.	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 10th day.
265 (L)...	31/ 3/35	2 c.cm.	"	3rd	3rd	104·0°F.	4·0°F.	Recovered.	8/ 5/35	2 c.cm.	Temperature reaction only. Recovered.
270 (L)...	31/ 3/35	2 c.cm.	"	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
251 (L)...	31/ 3/35	2 c.cm.	"	3rd	6th	102·2°F.	2·0°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 8th day.
316 (K)...	31/ 3/35	2 c.cm.	"	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
310 (K)...	31/ 3/35	2 c.cm.	"	No Reaction.					8/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
273 (K)...	31/ 3/35	2 c.cm.	"	No Reaction.					18/ 5/35	2 c.cm.	Died of Rinderpest on 9th day.
308 (K)...	18/ 5/35	2 c.cm.	"	1st	2nd	103·6°F.	2·6°F.	Recovered.	18/ 5/35	2 c.cm.	Died of Rinderpest on 11th day.

TABLE 7. (EXPERIMENT 7.)

To determine the immunizing properties of Rinderpest Goat Spleen Vaccine in the form of—

(a) 1 *per cent. emulsion in* 50 *per cent. Glycerine Saline.* *(b)* 1 *per cent. emulsion in Normal Saline.*

After Storage (1) for 5 days at 42° F.

(2) for 5 days at 82° F.

Cattle Number.	INJECTION OF VACCINE.			REACTION.					IMMUNITY TEST.		
	Date Injected.	Form of Vaccine.	Quantity.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
399 (L).	19/8/35	1 per cent. emulsion in 50 per cent. Glycerine-Saline after storage for 5 days at 42° F.	2 c.cm.		No definite temperature reaction.				9/9/35	2 c.cm.	Slight temperature reaction. Recovered.
402 (L).	19/8/35	" "	2 c.cm.	3rd	4th	105·0°F.	3·0°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 99·2°F. to 101·4°F. Recovered.
395 (K)	19/8/35	" "	2 c.cm.	3rd	5th	103·6°F.	2·4°F.	Recovered.	9/9/35	2 c.cm.	Mild temperature reaction. Recovered.
357 (K)	19/8/35	" "	2 c.cm.	2nd	3rd	105·4°F.	4·6°F.	Recovered.	9/9/35	2 c.cm.	Immune.
408 (L).	19/8/35	1 per cent. emulsion in Normal Saline after storage for 5 days at 42°F.	2 c.cm.	1st	4th	106·0°F.	4·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
400 (L).	19/8/35	" "	2 c.cm.	6th	7th	102·2°F.	1·2°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 100°F. to 103°F. Recovered.
389 (K)	19/8/35	" "	2 c.cm.	3rd	6th	105·0°F.	4·0°F.	Recovered.	9/9/35	2 c.cm.	Mild temperature reaction. Recovered.
355 (K)	19/8/35	" "	2 c.cm.	2nd	3rd	104·8°F.	3·2°F.	Recovered.	9/9/35	2 c.cm.	Mild temperature reaction. Recovered.
404 (L).	19/8/35	1 per cent. emulsion in 50 per cent. Glycerine-Saline after storage for 5 days at 82°F.	2 c.cm.	3rd	5th	102·4°F.	1·2°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 99°F. to 105·4°F. Diarrhoea. Severe mouth lesions. Recovered.
409 (L).	19/8/35	" "	2 c.cm.		Mild temperature reaction.				9/9/35	2 c.cm.	Rise in temperature from 99·4°F. to 101·8°F. Recovered.
378 (K)	19/8/35	" "	2 c.cm.	3rd	5th	105·6°F.	5·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
356 (K)	19/8/35	" "	2 c.cm.	3rd	6th	104·6°F.	4·6°F.	Recovered.	9/9/35	2 c.cm.	Rise in temperature from 99·8°F. to 101·6°F. Recovered.
398 (L).	19/8/35	1 per cent. emulsion in Normal Saline after storage for 5 days at 82°F.	2 c.cm.	3rd	4th	103·6°F.	2·4°F.	Recovered.	9/9/35	2 c.cm.	Immune.
407 (L).	19/8/35	" "	2 c.cm.	3rd	5th	105·4°F.	3·8°F.	Recovered.	9/9/35	2 c.cm.	Immune.
373 (K)	19/8/35	" "	2 c.cm.	3rd	5th	104·4°F.	3·4°F.	Recovered.	9/9/35	2 c.cm.	Immune.
375 (K)	19/8/35	" "	2 c.cm.		No reaction.				9/9/35	2 c.cm.	No reaction.
412 (L).	Control.								9/9/35	2 c.cm.	Rise in temperature from 101°F. to 103·2°F. on 7th day. Recovered.
383 (K)	Control.								9/9/35	2 c.cm.	Rise in temperature from 100°F. to 105°F. Diarrhoea. Mouth lesions. Died of Rinderpest on 9th day.

TABLE 8. (EXPERIMENT 8.)

To determine the viability at 42° F. of Goat Adapted Rinderpest Virus in the form of Powdered Spleen dried in vacuo over Calcium Chloride.

Goat Number.	INJECTION OF VIRUS.			REACTION.				
	Date Injected.	Weight of Powdered Spleen.	Period Stored at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
257..........	17/12/34	0·2 gram	20 days	2nd	3rd	104·6°F.	3°F.	Recovered.
274..........	6/ 1/35	0·2 gram	40 days	3rd	3rd	105·2°F.	3·4°F.	Recovered.
287..........	25/ 1/35	0·1 gram	60 days	2nd	7th	106°F.	5°F.	Died on 12th day.
297..........	16/ 2/35	0·1 gram	80 days	2nd	3rd	105°F.	3°F.	Died on 29th day.
303..........	14/ 3/35	0·1 gram	102 days	3rd	3rd	103·8°F.	1·8°F.	Died on 11th day.
335..........	1/ 4/35	0·01 gram	120 days	4th	6th	105°F.	3°F.	Recovered.
337..........	1/ 4/35	0·1 gram	120 days	2nd	5th	105·2°F.	4·2°F.	Died on 7th day.
357..........	22/ 4/35	0·1 gram	141 days	3rd	7th	105°F.	2°F.	Died on 11th day.
355..........	14/ 5/35	0·1 gram	163 days	6th	15th	104·8°F.	2·6°F.	Died on 17th day.
369..........	12/ 6/35	0·1 gram	192 days	1st	4th	106·2°F.	4·2°F.	Recovered.
356..........	2/ 7/35	0·1 gram	211 days	6th	10th	105°F.	4·2°F.	Died on 17th day.
379..........	22/ 7/35	0·1 gram	231 days	8th	18th	105°F.	2·6°F.	Recovered.

TABLE 9. (EXPERIMENT 9.)

To determine the viability at 85° to 89° F. of Goat Adapted Rinderpest Virus in the form of powdered spleen dried in vacuo over Calcium Chloride.

Goat Number.	INJECTION OF VIRUS.			REACTION.				
	Date Injected.	Weight of Powdered Spleen.	Period Stored at 85° to 89°F. after two days at 42°F.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in Temperature.	Result.
1	2	3	4	5	6	7	8	9
317..........	11/ 4/35	0·01 gram	3 days	3rd	5th	103°F.	1·4°F.	Died on 16th day.
348..........	11/ 4/35	0·005 gram	3 days	4th	12th	104·4°F.	2·2°F.	Recovered.
352..........	14/ 4/35	0·005 gram	6 days	3rd	3rd	106°F.	3·4°F.	Died on 26th day.
349..........	14/ 4/35	0·01 gram	6 days	3rd	3rd	106°F.	4°F.	Died on 10th day.
351..........	14/ 4/35	0·005 gram	6 days	2nd	5th	104°F.	2°F.	Recovered.
333..........	14/ 4/35	0·01 gram	6 days	2nd	3rd	105°F.	3·2°F.	Died on 6th day.
350..........	18/ 4/35	0·01 gram	10 days	2nd	3rd	104°F.	0·8°F.	Died on 9th day.
326..........	18/ 4/35	0·01 gram	10 days	2nd	3rd	106°F.	3°F.	Recovered.
308..........	21/ 4/35	0·01 gram	13 days	2nd	7th	104°F.	3°F.	Recovered.
342..........	21/ 4/35	0·01 gram	13 days	Temperature	reaction	indefinite.		Died on 36th day.

TABLE 10. (EXPERIMENT 10.)

To determine the viability and immunizing properties of Goat Spleen Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride and then made into an emulsion with Normal Saline and stored for (a) 0 days, (b) 4 days at 42° F., (c) 4 days at 79° to 81° F.

Cattle	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Day of Initial Rise.	Day Peak Reached	Peak tempera-ture.	Rise in tem-perature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11	12
367 (K)..	27/ 7/35	0·1 gram	0 days........	2nd	3rd	104·8°F.	4·8°F.	Recovered.	16/ 8/35	2 c.cm.	Immune.
350 (K)..	27/ 7/35	0·1 gram	0 days........	2nd	3rd	103°F.	3°F.	Recovered.	16/ 8/35	2 c.cm.	Immune.
394 (K)..	5/ 8/35	0·02 gram	4 days at 42°F.	5th	7th	105·2°F.	5·2°F.	Recovered.	9/ 9/35	2 c.cm.	Immune.
345 (L)..	5/ 8/35	0·02 gram	4 days at 42°F.			No reaction.			9/ 9/35	2 c.cm.	Immune.
349 (K)..	5/ 8/35	0·02 gram	4 days at 79° to 81°F.	4th	5th	105°F.	5°F.	Died on 35th day	—	—	Died of intercurrent disease.
346 (L)..	5/ 8/35	0·02 gram	4 days at 79° to 81°F.	9th	13th	105°F.	4°F.	Recovered	9/ 9/35	2 c.cm.	Immune.
391 (K)..	Control to Nos. 367 and 350.								16/ 8/35	2 c.cm.	Temperature rise of 5°F. Destroyed on 5th day for removal of spleen.
383 (K)..	Control to Nos. 394, 345 and 346.								9/ 9/35	2 c.cm.	Died of Rinderpest on 9th day.

TABLE 11. (EXPERIMENT 11.)

To determine the immunizing properties of Rinderpest Goat Spleen Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride after storage for (1) 5 days at 42° F., (2) 5 days at 82° F.

Cattle Number.	INJECTION OF VACCINE.			REACTION.					IMMUNITY TEST.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Day of Initial Rise.	Day Peak Reached.	Peak Temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
401 (L)..	19/8/35	0·005 gram	5 days at 42°F.	3rd	4th	104·0°F.	2·6°F.	Recovered.	9/9/35	2 c.cm.	Immune.
406 (L)..	19/8/35	0·005 gram	,, ,,	3rd	5th	104·4°F.	3·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
387 (K)..	19/8/35	0·005 gram	,, ,,	3rd	5th	105·0°F.	3·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
362 (K)..	19/8/35	0·005 gram	,, ,,	3rd	5th	105·2°F.	4·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
405 (L)..	19/8/35	0·005 gram	5 days at 82°F.	5th	6th	103·2°F.	2·0°F.	Recovered.	9/9/35	2 c.cm.	Immune.
403 (L)..	19/8/35	0·005 gram	,, ,,	3rd	5th	102·6°F.	0·8°F.	Recovered.	9/9/35	2 c.cm.	Immune.
381 (K)..	19/8/35	0·005 gram	,, ,,	3rd	6th	105·4°F.	5·4°F.	Recovered.	9/9/35	2 c.cm.	Immune.
384 (K)..	19/8/35	0·005 gram	,, ,,	3rd	3rd	105·2°F.	3·6°F.	Recovered.	9/9/35	2 c.cm.	Immune.
412 (L)..	Control.								9/9/35	2 c.cm.	Rise in temperature from 101°F. to 103·2°F. on 7th day. Recovered.
383 (K)..	Control.								9/9/35	2 c.cm.	Died of Rinderpest on 9th day.

TABLE 12. (EXPERIMENT 12.)

To determine the effect of storage at 97° F. on the viability and immunizing properties of Goat Adapted Rinderpest Virus in the form of powdered spleen dried in vacuo over Calcium Chloride.

Cattle Number.	INJECTION OF VIRUS.			REACTION.					IMMUNITY TEST.		
	Date Injected.	Weight of Dried Spleen.	Period Stored at 97°F after four days at 42°F.	Day of Initial Rise.	Day, Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11	12
441 (K)..	12/12/35	0·005 gram	3 days	2nd	9th	103°F.	3°F.	Recovered.	26/1/36	2 c.cm.	Immune.
440 (K)..	12/12/35	0·01 gram	3 days	2nd	5th	105°F.	5°F.	Recovered.	26/1/36	2 c.cm.	Immune.
434 (K)..	12/12/35	0·01 gram	3 days	2nd	4th	102·6°F.	3°F.	Recovered.	26/1/36	2 c.cm.	Immune.
439 (K)..	15/12/35	0·005 gram	6 days	3rd	4th	101°F.	1·4°F.	Recovered.	26/1/36	2 c.cm.	Rise in temperature of 4·2°F. Diarrhoea. Severe mouth lesions Recovered.
479 (K)..	12/12/35	0·01 gram	6 days	4th	6th	103°F.	3°F.	Recovered.	26/1/36	2 c.cm.	Immune.
467 (K)..	12/12/35	0·01 gram	6 days	Mild indefinite temperature reaction.					26/1/36	2 c.cm.	Died of Rinderpest on 13th day.
450 (K)..	Control.								26/1/36	2 c.cm.	Rise in temperature of 4·8°F. Peak reached on 6th day. Destroyed on 6th day for removal of spleen.
477 (K)..	Control.								26/1/36	2 c.cm.	Rise in temperature of 4·6°F. Diarrhoea. Severe mouth lesions. Recovered.

TABLE 13. (EXPERIMENT 13.)

To determine the optimum dose of Goat Adapted Rinderpest Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride.

Cattle Number.	INJECTION OF VACCINE.		REACTION.					IMMUNITY TEST.		
	Date Injected.	Weight of Dried Spleen.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date of Immunity Test.	Quantity of Virulent Blood.	Reaction.
1	2	3	4	5	6	7	8	9	10	11
476 (K)..	3/1/36	0·1 gram	3rd	5th	103°F.	3·6°F.	Recovered	26/1/36	2 c.cm.	Immune.
460 (K)..	3/1/36	0·1 gram	3rd	3rd	103·2°F.	4·2°F.	Recovered.	26/1/36	2 c.cm.	Immune.
436 (K)..	3/1/36	0·01 gram	4th	7th	101·4°F.	2·4°F.	Recovered	26/1/36	2 c.cm.	Immune.
433 (K)..	3/1/36	0·01 gram	4th	5th	102·4°F.	3·4°F.	Recovered.	26/1/36	2 c.cm.	Immune.
438 (K)..	3/1/36	0·005 gram	Indefinite temperature reaction.					26/1/36	2 c.cm.	Immune.
468 (K)..	3/1/36	0·005 gram	3rd	6th	103·2°F.	4·2°F.	Recovered	26/1/36	2 c.cm.	Immune.
442 (K)..	3/1/36	0·001 gram	4th	6th	103·6°F.	4·2°F.	Recovered.	26/1/36	2 c.cm.	Immune.
482 (K)	3/1/36	0·001 gram	4th	6th	102·4°F.	3°F.	Recovered.	26/1/36	2 c.cm.	Immune.
437 (K)..	3/1/36	0·0005 gram	5th	7th	102·0°F.	3·0°F.	Recovered	26/1/36	2 c.cm.	Immune.
481 (K)..	3/1/36	0·0005 gram	5th	6th	102·0°F.	3·0°F.	Recovered.	26/1/36	2 c.cm.	Immune.
450 (K)..	Control.							26/1/36	2 c.cm.	Rise in temperature of 4·8°F. Peak reached on 6th day. Destroyed on 6th day for removal of spleen.
477 (K)..	Control.							26/1/36	2 c.cm.	Rise in temperature of 4·6°F. Diarrhoea. Severe mouth lesions. Recovered.

TABLE 14. (EXPERIMENT 14.)

To determine the viability, under adverse field conditions, of Goat Adapted Rinderpest Virus in the form of powdered goat spleen dried in vacuo over Calcium Chloride.

Method.—The virus was packed in ice in a Thermos Jar which was stored at 99° F. The ice was replenished on the fourth day (when the temperature in the jar was 56° F.) and the flask replaced in the incubator at 97° F. The testing of the virus was commenced on the 6th day after ice was last put in the flask, i.e. on the 9th day after it was first packed in the Thermos Jar.

Cattle Number.	INJECTION OF VIRUS.			REACTION.					IMMUNITY TEST.		
	Date Injected.	Weight of Dried Spleen.	Number of days Thermos Stored at 97°F after last filled with Ice.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
513 (K)..	10/7/36	0·01 gram	Six..........	4th	5th	105·4°F.	5·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
518 (K)..	10/7/36	0·01 gram	Six..........	3rd	5th	103·6°F.	3·4°F.	Recovered.	11/8/36	2 c.cm.	Immune.
521 (K)..	10/7/36	0·001 gram	Six..........	3rd	6th	103·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
505 (K)..	10/7/36	0·001 gram	Six..........	3rd	5th	103·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
509 (K)..	12/7/36	0·01 gram	Eight.........	4th	4th	104·4°F.	4·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
517 (K)..	12/7/36	0·01 gram	Eight.........	3rd	4th	102·2°F.	2·2°F.	Recovered.	11/8/36	2 c.cm.	Died of Rinderpest on 9th day.
512 (K)..	12/7/36	0·001 gram	Eight.........	3rd	7th	104·0°F.	3·8°F.	Recovered.	11/8/36	2 c.cm.	Mild temperature reaction. Recovered.
523 (K)..	12/7/36	0·001 gram	Eight.........		No	reaction.			11/8/36	2 c.cm.	Severe temperature reaction with diarrhoea and mouth lessions. Recovered.
515 (K)..	14/7/36	0·01 gram	Ten.........		No	reaction.					Severe temperature reaction with diarrhoea and mouth lessions. Recovered.

TABLE 14—(*continued*).

Cattle Number.	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Number of days Thermos Stored at 97°F after last filled with Ice.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
520 (K)..	14/7/36	0·01 gram	Ten..........	3rd	7th	104·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
511 (K)..	14/7/36	0·001 gram	Ten..........		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 11th day.
524 (K)..	14/7/36	0·001 gram	Ten..........		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 9th day.
500 (K)..	16/7/36	0·01 gram	Twelve.......		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 10th day.
507 (K)..	16/7/36	0·01 gram	Twelve.......		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 9th day.
489 (K)..	16/7/36	0·001 gram	Twelve.......		No	reaction.			11/8/36	2 c.cm.	Severe temperature reaction with diarrhoea and mouth lesions. Recovered.
508 (K)..	16/7/36	0·001 gram	Twelve.......		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 12th day.
493 (K)..	18/7/36	0·01 gram	Fourteen......		No	reaction.			11/8/36	2 c.cm.	Died of Rinderpest on 12th day.

TABLE 14—(*continued*).

Cattle Number.	Injection of Virus.			Reaction.					Immunity Test.		
	Date Injected.	Weight of Dried Spleen.	Number of days Thermos Stored at 97°F after last filled with Ice.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
496 (K)..	18/7/36	0·01 gram	Fourteen......		No reaction.				11/8/36	2 c.cm.	Died of Rinderpest on 12th day.
490 (K)..	18/7/36	0·001 gram	Fourteen......		No reaction.				11/8/36	2 c.cm.	Died of Rinderpest on 10th day.
495 (K)..	18/7/36	0·001 gram	Fourteen......		No reaction.				11/8/36	2 c.cm.	Severe temperature reaction with diarrhoea and mouth lesions. Recovered.
506 (K)..	10/7/36	0·01 gram	Not stored in Thermos Jar. Control for stored virus	3rd	6th	103·0°F.	3·0°F.	Recovered.	11/8/36	2 c.cm.	Immune.
510 (K)..	10/7/36	0·001 gram	,,	3rd	6th	102·0°F.	2·4°F.	Recovered.	11/8/36	2 c.cm.	Immune.
508 (K)..	Control for virus used in Immunity Test.								11/8/36	2 c.cm.	Died of Rinderpest on 13th day.
488 (K)..	Control for virus used in Immunity Test.								11/8/36	2 c.cm.	Died of Rinderpest on 10th day.

TABLE 15. (EXPERIMENT 15.)

To determine the duration of immunity conferred by Rinderpest Goat Spleen Vaccine in the form of powdered spleen dried in vacuo over Calcium Chloride.

Cattle Number.	INJECTION OF VACCINE.		REACTION.					IMMUNITY TEST.			
	Date Injected.	Weight of Dried Spleen.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Period Since Injection of Vaccine.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
453 (K)..	9/12/35	0·01 gram	3rd	5th	104·0°F.	4·2°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
445 (K)..	9/12/35	0·01 gram	2nd	5th	103·4°F.	3·4°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
480 (K)..	9/12/35	0·01 gram	3rd	5th	102·4°F.	2·6°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
444 (K)..	9/12/35	0·01 gram	3rd	5th	104·4°F.	5·4°F.	Recovered.	22/10/36	319 days	2 c.cm.	Initial rise in temperature on 10th day. Rise of 4·6°F. Recovered.
428 (L)..	9/12/35	0·01 gram	4th	7th	101·8°F.	2·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
429 (L)..	9/12/35	0·01 gram	4th	7th	101·8°F.	2·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
423 (L)..	9/12/35	0·01 gram	5th	7th	101·8°F.	1·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
422 (L)..	9/12/35	0·01 gram	3rd	6th	102·4°F.	3·0°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
419 (L)..	9/12/35	0·01 gram	3rd	5th	103·4°F.	4·0°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
418 (L)..	9/12/35	0·01 gram	3rd	6th	100·8°F.	1·8°F.	Recovered.	22/10/36	319 days	2 c.cm.	Immune.
469 (K)..	9/12/35	0·01 gram	3rd	5th	103·4°F.	4·4°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
458 (K)..	9/12/35	0·01 gram	3rd	3rd	103·0°F.	3·0°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
449 (K)..	9/12/35	0·01 gram	2nd	4th	104·2°F.	4·0°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Initial rise in temperature on 5th day. Rise of 3·0°F. Recovered.
432 (L)..	9/12/35	0·01 gram	3rd	8th	102·2°F.	2·2°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
430 (L)..	9/12/35	0·01 gram	5th	5th	103·0°F.	3·0°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.
414 (L)..	9/12/35	0·01 gram	3rd	5th	104·4°F.	4·4°F.	Recovered.	20/ 5/37	429 days	2 c.cm.	Immune.

TABLE 15—(*continued*).

Cattle Number.	Injection of Vaccine.		Reaction.					Immunity Test.			
	Date Injected.	Weight of Dried Spleen.	Day of Initial Rise.	Day Peak Reached.	Peak temperature.	Rise in temperature.	Result.	Date Injected.	Period Since Injection of Vaccine.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7	8	9	10	11	12
413 (L)..	9/12/35	0·01 gram	3rd	6th	104·0°F.	4·4°F.	Recovered.	20/5/37	429 days	2 c.cm.	Immune.
427 (L)..	9/12/35	0·01 gram		No	reaction.			20/5/37	429 days	2 c.cm.	Immune.
547 (K)..	Control.							20/5/37		2 c.cm.	Died of Rinderpest on 8th day.
544 (K)..	Control.							20/5/37		2 c.cm.	Died of Rinderpest on 8th day.
462 (K)..	9/12/35	0·01 gram	3rd	5th	104·0°F.	4·0°F.	Recovered.	1/1/38	755 days	2 c.cm.	Initial rise in temperature on 6th day. Rise of 2·0°F. Recovered.
472 (K)..	9/12/35	0·01 gram	3rd	5th	103·6°F.	4·2°F.	Recovered.	1/1/38	755 days	2 c.cm.	Initial rise in temperature on 7th day. Rise of 2·0°F. Recovered.
335 (L)..	9/12/35	0·01 gram	3rd	5th	100·8°F.	2·0°F.	Recovered.	1/1/38	755 days	2 c.cm.	Immune.
324 (L)..	9/12/35	0·01 gram		No	reaction.			1/1/38	755 days	2 c.cm.	Immune.
267 (L)..	9/12/35	0·01 gram	3rd	5th	100·0°F.	2·0°F.	Recovered.	4/1/38	755 days	2 c.cm.	Immune.
337 (L)..	9/12/35	0·01 gram	3rd	4th	100·2°F.	1·2°F.	Recovered.	4/1/38	755 days	2 c.cm.	Immune.
582 (K)..	Control.							4/1/38		2 c.cm.	Died of Rinderpest on 8th day.
562 (K)..	Control.							4/1/38		2 c.cm.	Rise in temperature of 4·6°F. Severe mouth lesions. Recovered

TABLE 16. (EXPERIMENT 16.)

To determine the time taken to develop immunity.

Cattle Number.	INJECTION OF VACCINE.		INJECTION OF VIRUS.		
	Date Injected.	Quantity of Dried Goat Spleen.	Interval after Injection of Vaccine.	Quantity of Dried Bovine Spleen.	Result.
1	2	3	4	5	6
583 (K)	5/2/38	0·0025 gram	24 hours..........	0·01 gram	Diarrhoea. Recovered.
558 (K)	5/2/38	0·00025 gram	24 hours..........	0·01 gram	A few small ulcers in mouth. Recovered.
580 (K)......................	5/2/38	0·0025 gram	36 hours..........	0·01 gram	No reaction. Immune.
571 (K)	5/2/38	0·00025 gram	36 hours..........	0·01 gram	Diarrhoea. Recovered.
557 (K)......................	5/2/38	0·0025 gram	48 hours	0·01 gram	No reaction. Immune.
586 (K)	5/2/38	0·00025 gram	48 hours..........	0·01 gram	No reaction. Immune.
567 (K)......................	5/2/38	0·0025 gram	60 hours.	0·01 gram	No reaction. Immune.
577 (K)......................	5/2/38	0·00025 gram	60 hours..........	0·01 gram	No reaction. Immune.
576 (K)......................	5/2/38	0·0025 gram	72 hours	0·01 gram	No reaction. Immune.
560 (K)......................	5/2/38	0·00025 gram	72 hours......	0·01 gram	No reaction. Immune.
569 (K)......................	Control.		Injected on 7/2/38..	0·01 gram	Died of Rinderpest on 9th day.
494 (K)......................	8/9/36	0·005 gram	3 days..........	0·01 gram	No reaction. Immune.
487 (K)......................	8/9/36	0·0025 gram	3 days..........	0·01 gram	No reaction. Immune.
485 (K)	8/9/36	0·005 gram	5 days..........	0·01 gram	No reaction. Immune.
533 (K)......................	8/9/36	0·0025 gram	5 days..........	0·01 gram	No reaction. Immune.
526 (K)......................	8/9/36	0·005 gram	7 days	0·01 gram	No reaction. Immune.
529 (K)......................	8/9/36	0·0025 gram	7 days..........	0·01 gram	No reaction. Immune.
519 (K)......................	8/9/36	0·005 gram	9 days	0·01 gram	No reaction. Immune.
484 (K)......................	8/9/36	0·0025 gram	9 days	0·01 gram	No reaction. Immune.
497 (K)......................	8/9/36	0·005 gram	11 days..........	0·01 gram	No reaction. Immune.
504 (K)......................	8/9/36	0·0025 gram	11 days..........	0·01 gram	No reaction. Immune.
501 (K)......................	Control.		Injected on 19/9/36.	0·01 gram	Died of Rinderpest on 14th day.

To determine the immunizing value of Goat Adapted Rinderpest virus in the form of powdered spleen dried in vacuo over Calcium Chloride and stored alternately for 12 hours at 80° F. and for 12 hours at 97° F.

Cattle Number.	INJECTION OF VACCINE.			IMMUNITY TEST.		
	Date Injected.	Weight of Dried Spleen.	Period Stored.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7
581 (K)......................	21/3/38	0·0025 gram	6 days.......	21/4/38	2 c.cm.	Pronounced temperature reaction. Recovered.
578 (K)......................	21/3/38	0·0025 gram	6 days.......	21/4/38	2 c.cm.	Died of Rinderpest on 13th day.
574 (K)......................	23/3/38	0·0025 gram	8 days.......	21/4/38	2 c.cm.	Died of Rinderpest on 9th day.
579 (K)......................	23/3/38	0·0025 gram	8 days.......	21/4/38	2 c.cm.	Diarrhoea. Mouth lesions. Recovered.
566 (K)......................	25/3/38	0·0025 gram	10 days.......	21/4/38	2 c.cm.	Died of Rinderpest on 9th day.
570 (K)......................	25/3/38	0·0025 gram	10 days.......	21/4/38	2 c.cm.	Diarrhoea. Mouth lesions. Recovered.
568 (K)......................	27/3/38	0·0025 gram	12 days.......	21/4/38	2 c.cm.	Died of Rinderpest on 14th day.
569 (K)......................	27/3/38	0·0025 gram	12 days.......	21/4/38	2 c.cm.	Died of Rinderpest on 14th day.
587 (K)......................	28/3/38	0·0025 gram Control	Vaccine stored for 14 days at 42°F.	21/4/38	2 c.cm.	Immune.
594 (K)......................	28/3/38	0·0025 gram		21/4/38	2 c.cm.	Immune.
603 (K)......................	Virus control			21/4/38	2 c.cm.	Died of Rinderpest on 10th day.

TABLE 18. (EXPERIMENT 18.)

To determine the effect of storage at 37° C. on Bennett's Glycerine-Vaccine.

Cattle Number.	INJECTION OF VACCINE.			IMMUNITY TEST.		
	Date Injected.	Weight of Spleen Pulp Injected.	Period Stored at 37°C.	Date Injected.	Quantity of Virulent Blood.	Result.
1	2	3	4	5	6	7
559 (K)......................	25/3/38	1 gram	4 days	21/4/38	2 c.cm.	Died of Rinderpest on 11th day.
584 (K)......................	25/3/38	1 gram	4 days	21/4/38	2 c.cm.	Died of Rinderpest on 13th day.
588 (K)......................	27/3/38	1 gram	6 days	21/4/38	3 c.cm.	Diarrhoea. Mouth lesions. Recovered.
593 (K)......................	27/3/38	1 gram	6 days	21/4/38	2 c.cm.	Died of Rinderpest on 11th day.
597 (K)......................	5/4/38	1 gram	15 days	21/4/38	2 c.cm.	Diarrhoea. Mouth lesions. Recovered.
595 (K)......................	5/4/38	1 gram	15 days	21/4/38	2 c.cm.	Died of Rinderpest on 10th day.
600 (K)......................	10/4/38	1 gram	20 days	21/4/38	2 c.cm.	Died of Rinderpest on 11th day.
598 (K)......................	10/4/38	1 gram	20 days	21/4/38	2 c.cm.	Died of Rinderpest on [illegible] day.

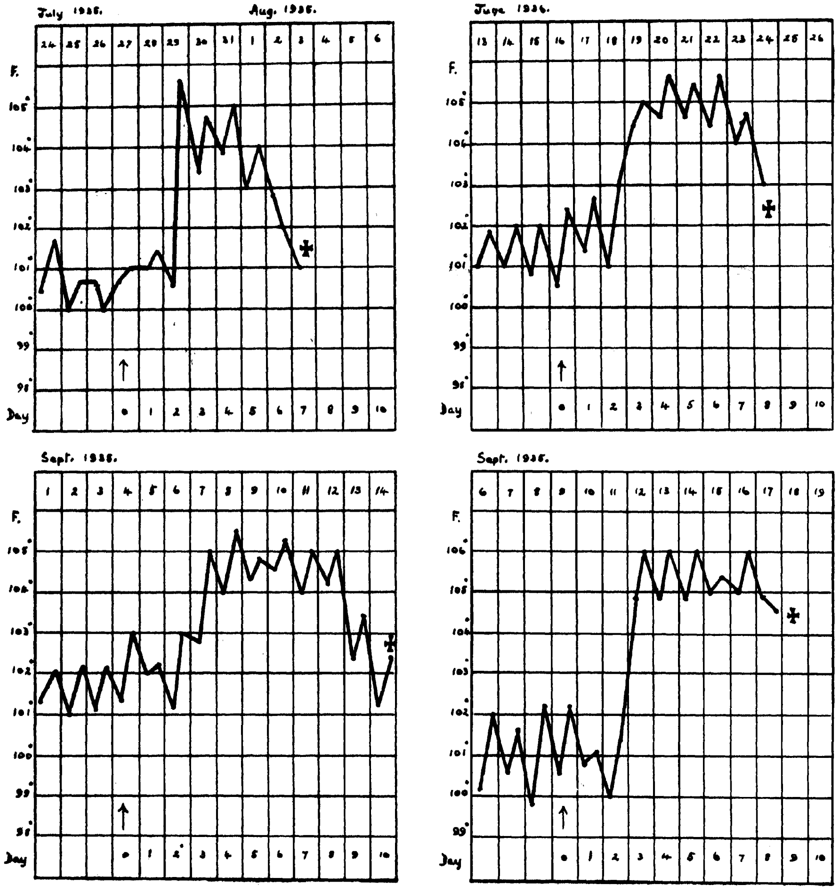

Figure 1.—Four typical temperature charts of cattle used in the passage of the Kungyangon cattle virus.

↑ = Injection of virus. ✝ = Died of rinderpest.

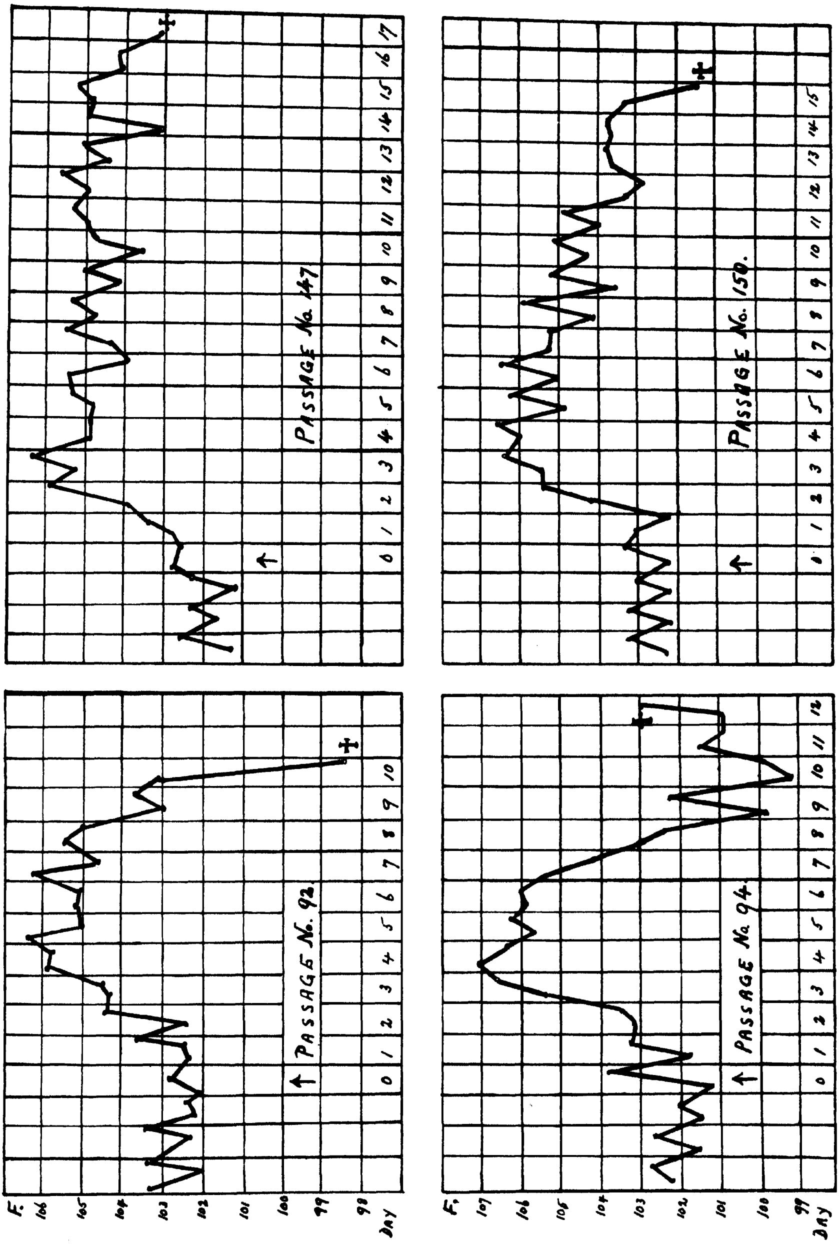

Figure 2.—Four typical temperature charts of goats used in the passage of the goat adapted rinderpest virus.

↑ =Injection of virus. † = Died of rinderpest.

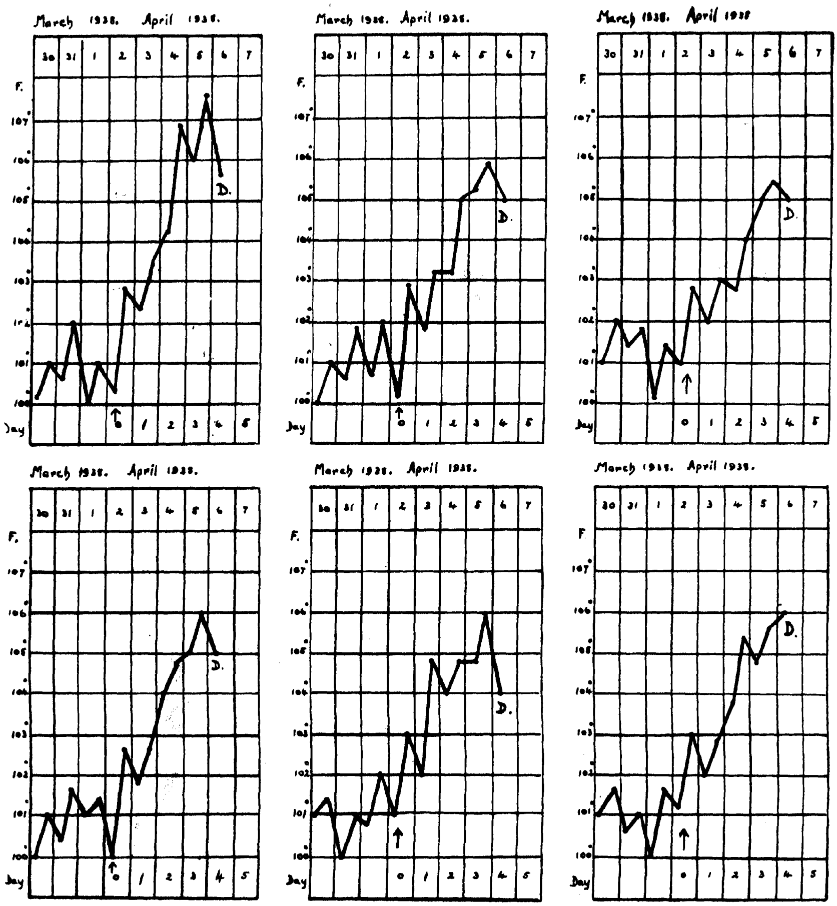

Figure 3.—Six typical temperature charts of a batch of goats used in the preparation of dried goat spleen vaccine.

↑ = Injection of virus. D = Destroyed.

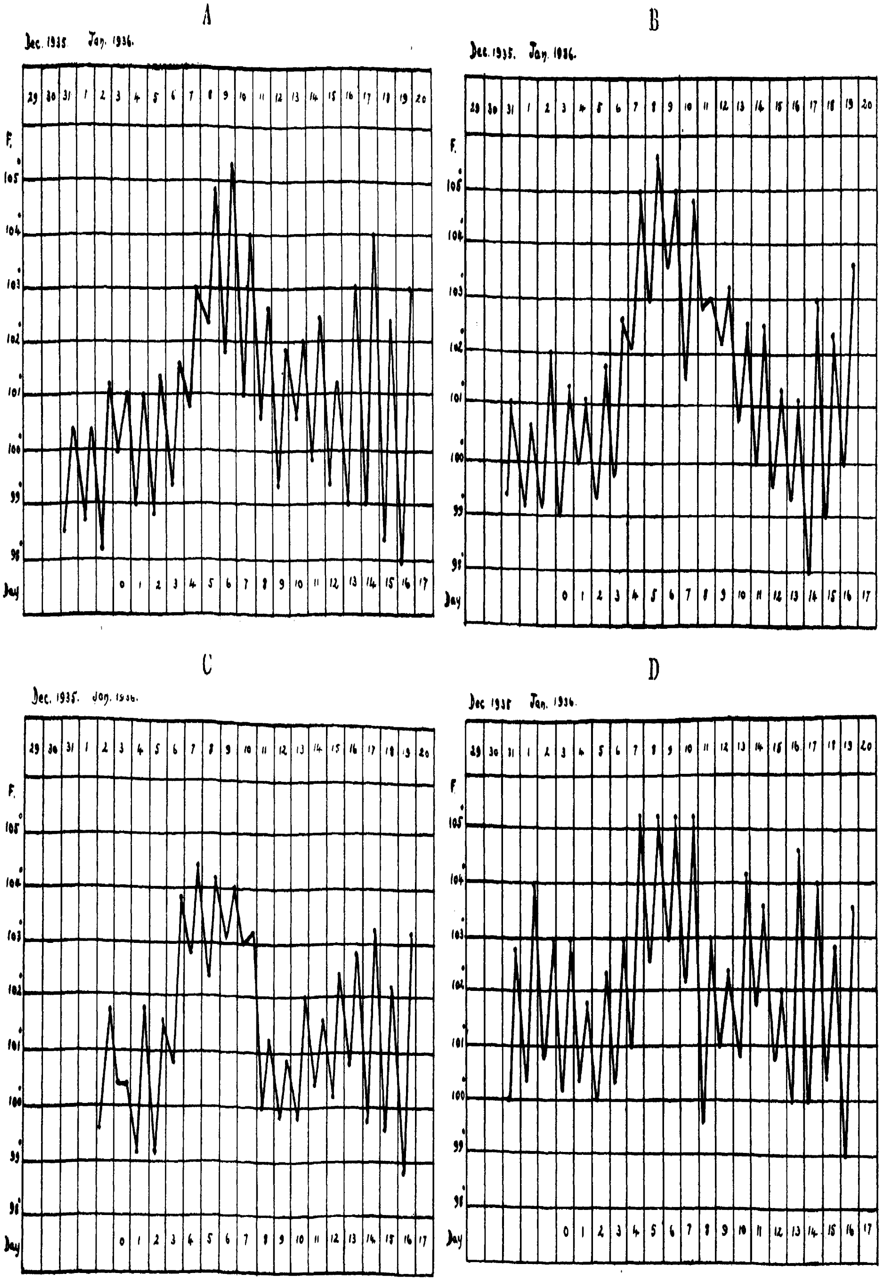

Figure 4.—Four typical temperature charts of Kyaukpyu cattle injected with dried goat spleen vaccine.

A = Bovine 433. Injected 0·01 gr. dried vaccine.
B = Bovine 442. Injected 0·001 gr. dried vaccine.
C = Bovine 468. Injected 0·005 gr. dried vaccine.
D = Bovine 481. Injected 0·0005 gr. dried vaccine.

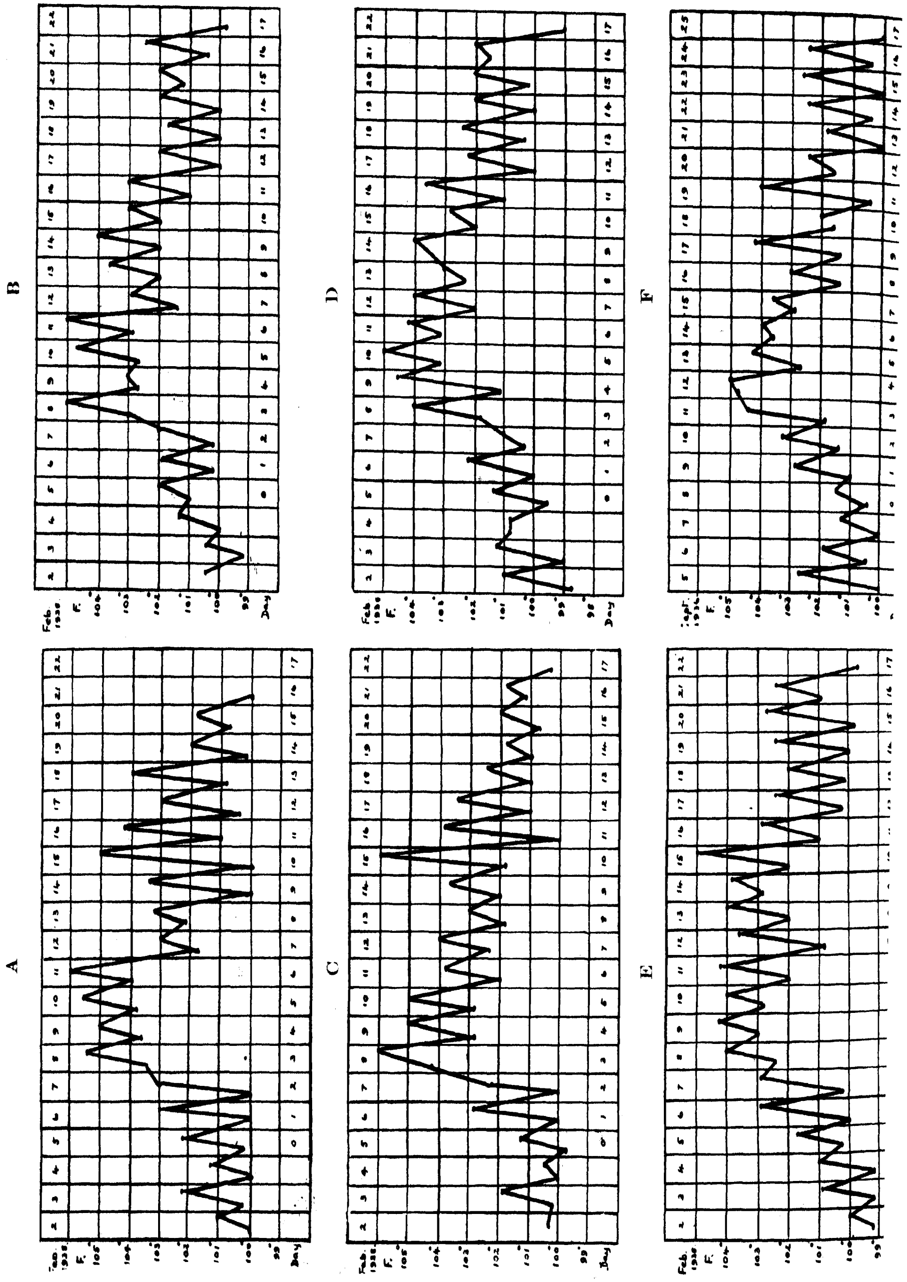

Figure 5.—Temperature charts of six Kyaukpyu cattle given cattle virus soon after the injection of dried spleen vaccine to determine the onset of immunity (Expt. 16).

A=Bovine 583. Virus given 24 hours after vaccine.
B=Bovine 567. Virus given 60 hours after vaccine.
C=Bovine 580. Virus given 36 hours after vaccine.

D=Bovine 576. Virus given 72 hours after va
E=Bovine 557. Virus given 48 hours after
F=Bovine 485. Virus given 120 hours after

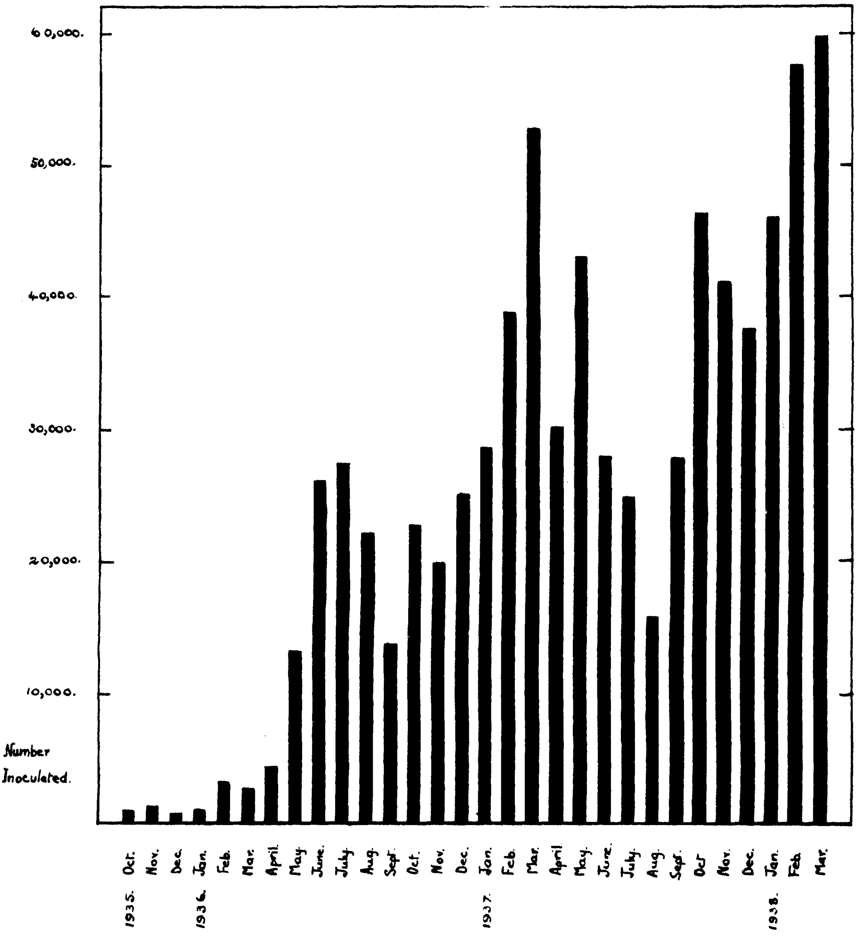

Figure 6.—Number of cattle inoculated with dried goat spleen vaccine from October, 1935, to March, 1938.

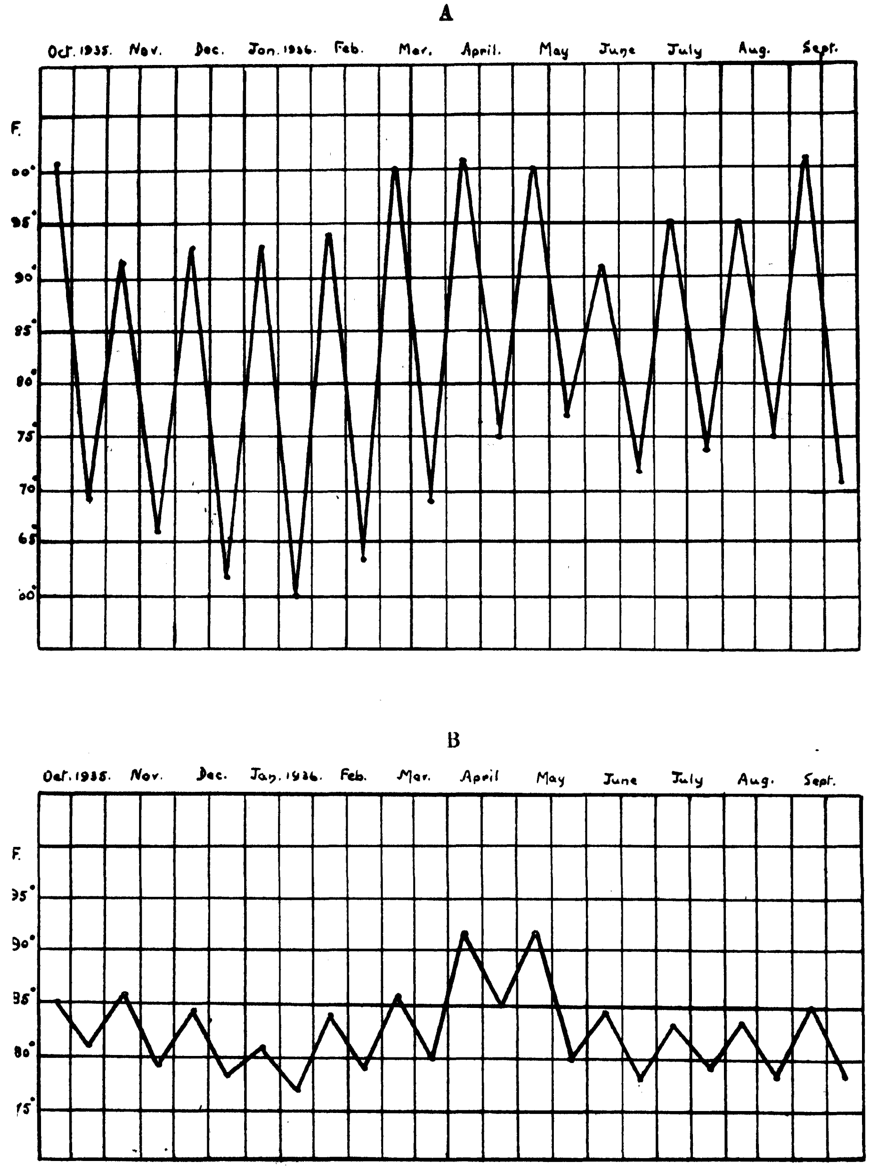

Figure 7.—Chart showing the monthly maximum and minimum room and shade temperatures at the Veterinary Research Laboratory, Insein, over one year.

A = Shade temperature.
B = Room temperature

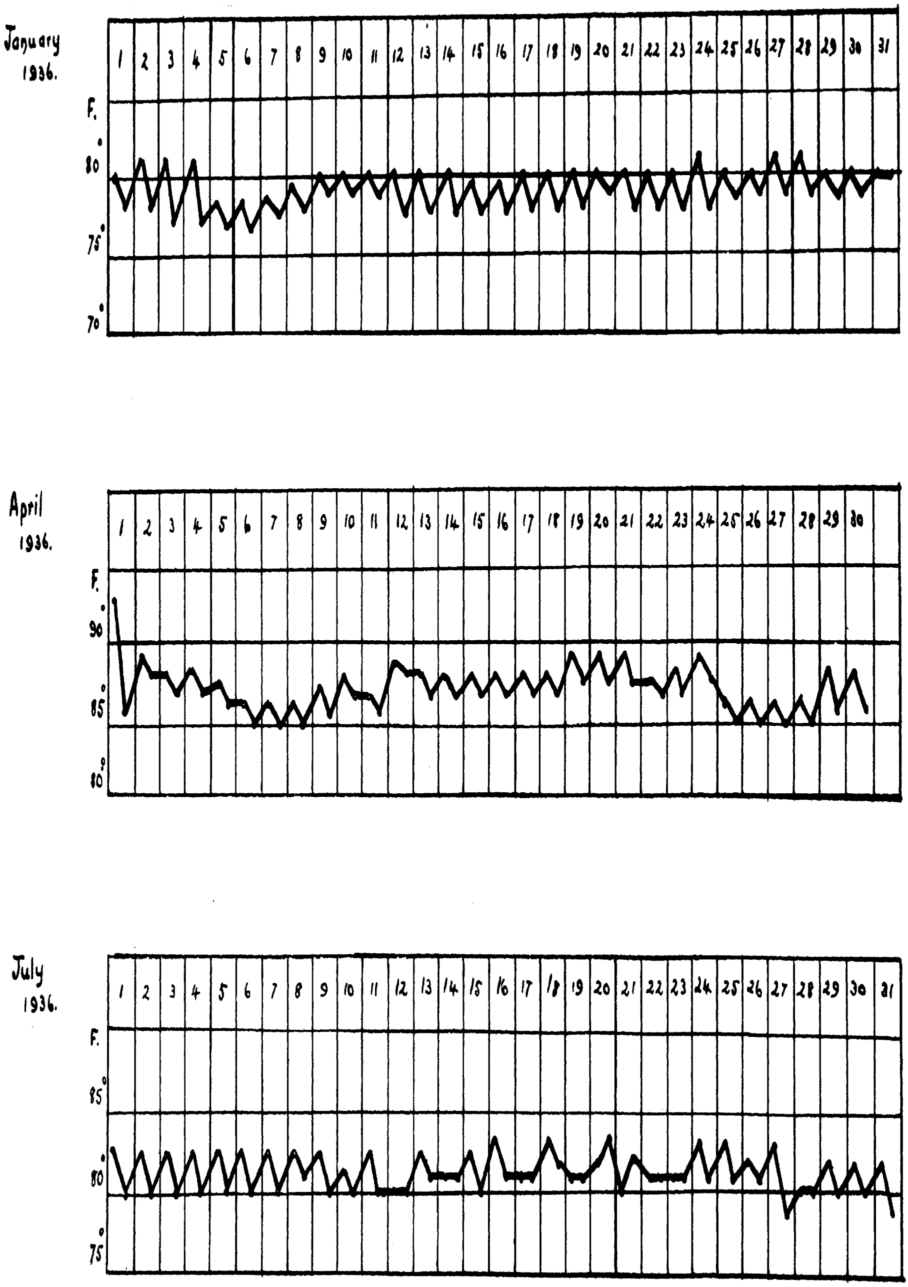

Figure 8.—Chart showing the daily maximum and minimum room temperatures at the Veterinary Research Laboratory, Insein, in January (cold weather), April (hot weather), and July (rainy weather).

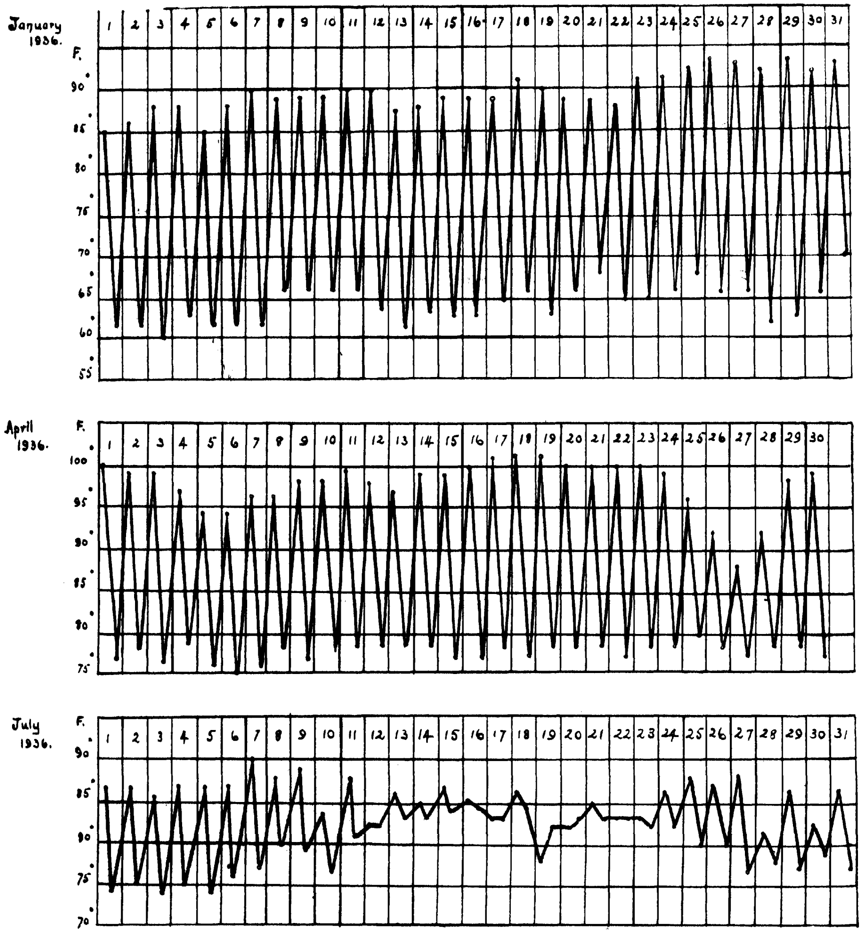

Figure 9.—Chart showing the daily maximum and minimum shade temperatures at the Veterinary Research Laboratory, Insein, in January (cold weather), April (hot weather), and July (rainy weather).

Section II.

Bacteriology.

Mason, J. H. and Robinson, E. M. The isolation of *Cl. Welchii*, Type B. from foals affected with dysentery 333

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The Isolation of *Cl. Welchii*, Type B, from Foals affected with Dysentery.

By J. H. MASON, Section of Protozoology and Virus Diseases, and E. M. ROBINSON, Section of Bacteriology, Onderstepoort.

To our knowledge the only reference to the occurrence of "lamb dysentery" (bloedpens) in foals is that of Montgomerie and Rowlands (1937). They record that the only abnormality seen in the foal they examined was "an acute congestion of the small intestine throughout its length with numerous discrete ulcers of the mucous membrane". From these ulcers they succeeded in isolating *Cl. welchii*, Type B. We have had one experience of the same disease in foals and the following communication summarizes our findings.

History of the Disease.

The two outbreaks of which we have knowledge occurred in the foals of a thoroughbred stud farm in the Cape Province, in an area in which lamb dysentery is present, but although about 30 ewes were running on this farm none of their progeny died of the disease nor could a previous history of the illness be elicited.

Apparently the disease made its appearance on this particular farm in 1936, and at the time of the field investigation it was stated that a like condition had not been seen on other stud farms of the district; however, inquiries made subsequently elicited the information that it had occurred occasionally.

The 1936 Outbreak.

Forty-three foals were born in the season and seven died of dysentery. In five instances the mares were from 9 to 23 days overdue and in two cases they foaled at full term; thus late foaling was not necessarily a factor in the causation of the disease. The foal of the nineteenth dam was the first to be infected, after which cases occurred right up to the end of the season. Foaling took place in loose boxes, the floors of which were covered with dry powdery horse dung, over which, in some instances, straw was spread.

The 1937 Outbreak.

The outbreak commenced at the beginning of the foaling season and at the time of the visit of one of us (E.M.R.), 7 of 17 foals had died. One would have been justified in predicting a 50 per cent. morbidity rate in the foals of the 26 mares which remained to foal if no preventive measures were taken. As in the 1936 outbreak, some mares carried their foals over time, but this appeared to have no etiological significance; all were in good condition and had adequate milk. The in-foal dams were kept in a large, bare camp and daily received hay thrown on the ground. When due to foal they were transferred to a small enclosure near loose boxes and allowed to foal there; on the ground of this enclosure a considerable amount of dung had been allowed to collect.

Symptoms.—In all the foals involved in the 1936 and 1937 outbreaks symptoms were noticed within 48 hours after birth and, as a rule death supervened within a further 24 hours; however, one affected foal lived for 5 days. Between the 24th and the 48th hour after birth the foal stopped sucking and appeared to be in pain; diarrhoea was marked, the faeces being very fluid in consistency and reddish-brown in colour. The animal gradually became weaker and died in a comatose state about 12 to 24 hours later. This description, given by the manager, was confirmed in a foal seen by one of us during the field investigation; it may be added that the temperature was normal and that, just before death, the breathing was very rapid.

Pathological Changes.—Two foals which had died of dysentery were autopsied, and, in both, the only macroscopic lesions noticed were in the alimentary tract. The small intestine was markedly hyperaemic, the wall showing a number of dark red spots (?-commencing ulcers) up to a centimetre in diameter. These were more frequent in the ileum than in any other portion of the small gut, but at no level was actual ulceration present. The caecum and colon were only slightly hyperaemic and the intestinal contents, from the small intestine to the rectum, were fluid in consistency and dark red in colour.

THE ISOLATION OF *Cl. welchii*, TYPE B.

The blood and internal organs yielded, in artificial culture, no germs of a pathogenic nature and, although a special search was made for paratyphoid bacilli, none were found. Another probable cause was a member of the *Cl. welchii* group of anaerobic organisms, and from the small gut contents of two foals we succeeded in isolating such a germ.

Foal 1.

The supernatant fluid of the centrifuged intestinal contents was lethal for mice (intravenous injection) in a dose of 0·01 cc. Type A antitoxin, type B, 1930 variety antitoxin (i.e. containing the alpha and beta but not the epsilon fraction) and type D antitoxin had no apparent neutralizing effect on this material; type B antitoxin 1922 variety (i.e. containing the epsilon fraction in addition to the alpha and beta) and a mixture of types B, 1930 variety and D antitoxins neutralized the toxin contained in the supernatant.

By appropriate methods, a pure culture of a welch-like germ was isolated, and toxins were prepared by growing it in donkey-flesh meat-broth for one and for five days respectively. Antiserum was prepared by hyperimmunizing a goat with the one-day toxin.

Morphologically and culturally the culture isolated could not be distinguished from known strains of *Cl. welchii*, type B. Its fermentative reactions were as follows:—Acid and gas production in saccharose, galactose, lactose, laevulose, and sorbite; acid without gas in glucose, maltose, and glycerine (slight); and no reaction in mannite, dulcite, and inulin; gelatin and Loeffler's serum medium were liquefied, inspissated horse serum was pitted but not liquefied and litmus milk reacted with a " stormy fermentation ". The post-mortem picture in a guinea-pig killed by the intramuscular inoculation of 1·0 cc. of an 18 hours' broth culture was, in all respects, similar to that produced by *Cl. welchii*, type B.

Table 1 records the results obtained by titrating the one-day toxin (precipitated with ammonium sulphate and dried) against various antitoxins.

TABLE 1.

Amount of Various Antitoxins Required to Neutralize the One-day Toxin.

(M.L.D. of toxin = 0·002—0·003 cc.)

Neutralizing dose of antitoxin (cc.).

Toxin (cc.).	Type A GG 2748.	Type B 1930 variety. 1789.	Type B 1922 variety. C2540.	Type B OP.	Type D Ex 15.	Foal.
0·005............	>0·1	—	—	—	—	—
0·1..............	—	>0·1	0·02	0·04	—	0·04
0·025............	—	0·0015	—	—	—	—
0·003............	—	—	—	—	>0·1	—

(G.G.2748, C.2540 and Ex 15 were obtained from the Wellcome Laboratories; C.2540 was a current issue of lamb dysentery antitoxin; type B, OP was a mixture of type B 1930 variety and type D antitoxins made at Onderstepoort; " foal " antitoxin was made by hyperimmunizing a goat with the one-day foal culture toxin.)

These results show that the toxin was of the lamb dysentery or bloedpens bacillus type (see Mason, 1935). Types A and D antitoxins (0·1 cc. amounts) did not neutralize 1 M.L.D.; type B antitoxin (C.2540 0·02 cc.), containing the beta and the epsilon fractions, neutralized about 50 M.L.D.; 0·1 cc. of type B antitoxin (1789), devoid of the epsilon fraction, did not neutralize 50 M.L.D. but 0·0015 cc. was effective against about 10 M.L.D. Mice which received 0·1 cc. of a mixture of 0·5 cc. of toxin and 0·5 cc. of 1789 antitoxin died, whereas those receiving 0·05 cc. lived. A series of mixtures were made, each containing 0·1 cc. of toxin and 0·1 cc. of D (Ex 15) antitoxin and to each of these mixtures different amounts of 1789 antitoxin were added; under these conditions 0·003 cc. of the latter antitoxin produced neutrality. These experiments show that 0·1 cc. of toxin contained about 2 M.L.D. of the epsilon toxic fraction; if this portion was neutralized with a type D antitoxin, a relatively small amount of a non-anti-epsilon-containing type B serum was effective against a test dose of about 50 M.L.D. of the "foal" toxin.

In Table 2, the results obtained in titrating the five-day toxin (precipitated and dried) are given.

Table 2.

Amount of Various Antitoxins Required to Neutralize the Five-day Toxin.

(M.L.D. of toxin = 0·003–0·004 cc.)

Neutralizing dose of antitoxin.

Toxin (cc.).	Type A GG2748.	Type B 1930 variety. 1789.	Type B 1922 variety. C2540.	Type B OP.	Type D Ex 15.	Foal.
0·003	>0·1	>0·1	—	—	—	—
0·1	—	—	0·04	0·08	0·06	0·015

The results given above show the five-day toxin consisted, probably entirely, of the epsilon fraction. No titration to detect small amounts of the beta portion was conducted and the alpha fraction was not investigated.

The reciprocal toxin-antitoxin titration was carried out by using the one- and the five-day toxins of the bloedpens strain of *Cl. welchii*, type B, and an antitoxin made in a goat with the foal strain. One of us (Mason, 1935) has already shown that the bloedpens strain is serologically identical with the original lamb dysentery organism. The results were as follows:—0·1 cc.–0·15 cc. of serum neutralized 100 M.L.D. of the one-day toxin and 0·004 cc. neutralized 60 M.L.D. of the five-day toxin.

Foal 2.

The supernatant fluid of the spun contents of the small intestine was not lethal for mice, on intravenous injection, in a dose of 0·1 cc. A welch-like germ isolated from this material was identical morphologically and culturally, with that obtained from foal 1. A detailed serological examination of the toxin was not carried out but it was shown that a test dose of a one-day toxin, containing at least 10 M.L.D., was neutralized by type B antitoxin, 1922 variety (C.2540) and by a mixture of type B, 1930 variety (1789) and type D (Ex 15) antitoxins, but not by either separately.

PREVENTION.

Between visiting the farm and establishing a diagnosis 11 foals (Nos. 15-26) had been born and none had developed dysentery; foals 12 and 14 had died during the visit. Although anti-serum was sent off immediately the causation was known, only one foal received an injection and this, weak at birth, developed dysentery and died. One other foal (No. 37), not passively protected, succumbed to the disease. It is fortunate that the farm manager omitted to use the antitoxin because, if he had used it, we might have attributed the lowered disease incidence to the effect of the serum and not to other causes. It is possible that the recommendation to permit foaling only in the open resulted in a reduction in the losses from 7 out of the first 17 foals to 2 out of the following 26. However, it is probable that the infection was present (even if in less degree) in the foaling camp as well as in the boxes because the disease occurred in two foals which had not been near boxes.

SUMMARY.

An outbreak of dysentery in foals is recorded. The symptoms and post-mortem lesions have much in common with those of lamb dysentery. From the intestinal contents of two foals, *Cl. welchii*, type B (the lamb dysentery or bloedpens bacillus) was isolated.

ACKNOWLEDGMENT.

We have pleasure in thanking Dr. R. A. O'Brien, the Director, and Dr. R. F. Montgomerie, the Veterinary Superintendent, of the Wellcome Physiological Research Laboratories for the generous supply of lamb dysentery antitoxin.

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Section III.

Physiology.

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Studies on the Alimentary Tract of Merino Sheep in South Africa. IV.—Description of Experimental Technique.

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INTRODUCTION.

In the first two papers which appeared under this series (Mönnig and Quin, 1933, 1935), methods were described whereby the process of deglutition and the closure of the oesophageal groove was studied in Merino sheep. Various selected substances were dosed on to the back of the tongue of experimental animals and followed within short intervals by some indicator powder such as red oxide of mercury. All animals were slaughtered immediately after dosing, and the forestomachs, abomasum and intestines opened and examined for the presence of the indicator powder. By this procedure considerable information was gained as to the peculiarities of the oesophageal groove reflex and the route followed by materials in their passage along the digestive tract under varying circumstances.

In the third paper (Besselaar and Quin, 1935), the results were discussed of experimental bowel anastomosis in sheep. By this method, measured lengths of either large or small intestine were excluded from function through a short circuiting of the bowel. This afforded a means of assessing the significance of a decrease in the digestive and absorptive surface to general body economy.

As various other aspects of ruminant digestion are also under investigation, the present paper is devoted mainly to a description of some further experimental methods which the authors are adopting in the study of this subject.

Digestion in ruminant animals is admittedly a more complicated and more protracted process than that occurring in most of the other species of mammals, and consequently our knowledge in this respect is less complete than that concerned with the digestion of man or dog. Thus, very little is as yet known about the degree and significance of the predigestion of foodstuffs occurring within the forestomachs as a result of bacterial and possibly other types of fermentation when compared with the true digestion which follows in the abomasum and upper levels of the small intestine. Similarly little is known about the activity of the digestive juices secreted from the true stomach and intestines of ruminants in comparison with those, for example, from the dog, although in most text books it is assumed that the processes are identical. With regard to the fundamental factors concerned in absorption from, and excretion into the digestive tract of ruminants our knowledge is equally incomplete.

It was for the above reasons, therefore, that experiments were devised primarily with the object of obtaining a clearer insight into the functioning of the different levels of the digestive tract of ruminants. For this purpose well-grown, adult, wether Merino sheep were selected in the majority of experiments, although a few bovines were also included in some aspects of the work carried out.

EXPERIMENTAL PROCEDURE.—THE CREATION OF BOWEL FISTULAE.

As an experimental method, the extensive use of bowel and salivary fistulae was first introduced by Pavlov in his classical work on digestion which was conducted mainly in dogs. By this method, he was able to gain an insight into the various phases of digestion which up till then had been largely shrouded in mystery. To-day the fistula method is well established as a means of investigating the digestive tract, although it is still largely confined to such animals as the dog. Thus, in ruminants it has so far only found limited application except as a means of studying the motility of the rumen and other forestomachs. In such cases large open fistulae are usually created by uniting the incised rumen with the abdominal skin in a flank operation. Wester (1926) exclusively used this method in his well-known work on the motility of the forestomachs and the closure of the oesophageal groove in cattle. Likewise did Schalk and Amadon (1928) and also Bergman and Dukes (1926) utilize this procedure in their researches. Recently, Krzywanek and Quast (1936), working on sheep, reported good results by using the improved double method of Brüggemann (1935) as based on the original device suggested by Morat.

Apart from the above-mentioned fistula methods, various other procedures have from time to time been adopted. Thus Czepa and Stigler (1929), working on goats, followed the onward movement of contrast mixtures through the forestomachs and abomasum by means of Röntgen ray photography. A similar method was adopted by Magee (1932) in his studies.

A third method which has found favour with some workers consists in the opening of the abdominal cavity of narcotised experimental animals in a bath of saline raised to body temperature. This allows of a close visual inspection of the bowel movements as well as their recording by means of cinematography. Thus far Mangold and Klein (1927), and more recently Dukes and Sampson (1937), all working on sheep, have recorded the results obtained by this procedure.

The method adopted in our experiments consisted in the creation of " closed " fistulae at various levels of the digestive tract in contrast to the " open " fistula method as commonly used on ruminant animals. For this purpose various types of cylindrical fistula tubes, mounted with a flange at one end, have been experimented with, the shape and size of the tube depending on whether it was intended for use in the rumen, abomasum, small intestine or large intestine. Of the metal tubes used, both the aluminium, and the chromium plated brass tubes proved rather disappointing since after being in the bowel for some time, sloughing frequently followed corrosion of the tubes either by the acids or alkalis elaborated in the tract. At other times, again, electrolysis would result in the deposition of materials at the internal opening of the tube thereby blocking it up. Thus far the tube which has given the minimum amount of trouble, especially as used in the rumen and caecum, is one turned up in a lathe from a cylindrical block of ebonite and provided with a wide flange at one end. Such tubes, not being subject to corrosion and electrolysis, are found to remain in their proper position in the body more or less indefinitely (see photo No. 1—ebonite tube on extreme right). For use in the rumen, a tube 6 cm. long, with a bore of 1·5 cm. and a circular flange of 4 cm. diameter, has so far given very satisfactory results. Correspondingly smaller tubes of the same pattern are used in the abomasum and the caecum. For the small intestine, much narrower tubes are employed, these being mounted with an oval boat-shaped flange at one end. In each case a slight hollowing out of the flange, thus presenting it with a turned-up appearance, has been found to be advantageous.

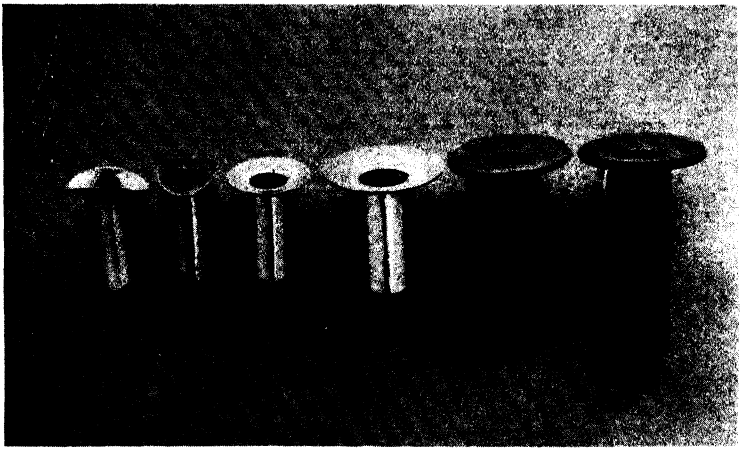

Photo No. 1.—Bowel fistula tubes of different types.

INSERTION OF THE FISTULA TUBE INTO THE BOWEL.

During a pre-period of 4 to 6 weeks, selected animals are penned off individually and kept on a constant ration of 360 grams crushed yellow maize and 300 grams hay daily. All animals are completely starved for 24-48 hours prior to operation, during which time the abdominal skin is clipped and shaved. The left flank is thus cleaned when a rumen fistula is to be inserted, whereas for all other intestinal fistulae the right flank is utilised. Abomasal fistulae, however, are brought out in the mid ventral position about 8 cm. posterior to the Xiphoid cartilage.

In each case general anaesthesia is induced through slow intravenous infusion of 10 per cent. choral hydrate in saline, narcosis being subsequently maintained with light ether inhalation, should this be necessary. Full aseptic precautions are observed throughout the operation, which comprises a laparotomy through either flank about 5 cm. behind and parallel to the last rib except in the case of an abomasal fistula, where a mid ventral opening is resorted to. For the creation of a rumen fistula the dorsal aspect of the dorsal ruminal sac is invariably selected. This part of the rumen, after being withdrawn through the abdominal wound as far as possible, is firmly caught up in a pair of long curved bowel forceps guarded with rubber tubing on both limbs. Using No. 1 gut on an eyeless needle, an oval purse string pattern is sutured through the muscular layer of a small selected part of the ruminal wall. See photo No. 1a.

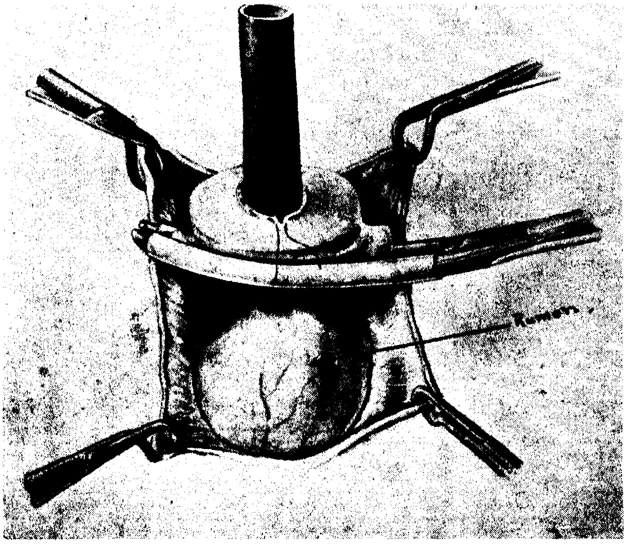

Photo No. 1 (*a*).—Diagrammatic sketch of a stage in ruminal fistula operation.

The ebonite tube, sterilised by boiling, is mounted with a short stout piece of rubber tubing at its thin end prior to its insertion in the rumen. A short slit is made through the rumen in the centre of the purse string suture through which the flange of the tube is then passed by slight stretching of the ruminal wound. This is followed by invagination of the lips and a tightening of the suture. Usually a second purse string suture is applied just above the first. After that, the free end of the tube is passed through a small slit made in the omentum which in this way is made to cover the ruminal incision. Next, a point on the skin is selected high up in the angle formed between the last rib and the transverse processes of the lumbar vertebrae. A stab wound is made at this point into the peritoneal cavity. By means of two pairs of long artery forceps passed through this wound, the rubber mounting on the fistula tube is securely gripped and the tube withdrawn through the stab opening as far as possible. This ensures a close contact between the rumen and the parietal peritoneum immediately around the flange of the tube. This contact is subsequently maintained by a collar of cotton wool tied down tightly round the tube between the rubber mounting and the skin. This is followed by closure of the laparotomy wound by three layers of continuous gut sutures one layer each for the peritoneum, muscle and skin respectively. The only dressing applied to the fresh wound is a thin film of collodion-iodoform. Healing in practically every instance follows by first intention. A firm circular adhesion is thereby established round about the fistula tube, usually no more than 6 cm. in diameter, uniting the rumen, omentum and parietal peritoneum within a few days. For the first three or four days following the operation, animals are usually kept on a light diet of green feed after which they are immediately returned to the standard laboratory ration of lucerne hay and maize, all animals being fed and watered individually in single pens. The tubes are kept closed by tight-fitting rubber stoppers inserted into the rubber mountings stretched over the ebonite tubes. Apart from periodic cleaning up of the outside of the tube, which may become slightly soiled, no other attention is required. Such tubes usually remain in position for an indefinite period. Thus several sheep carrying tubes for two years and over are included in the flock of fistula animals. (See photos Nos. 2 and 3.) Photo No. 4 is that of a bovine with an open ruminal fistula.

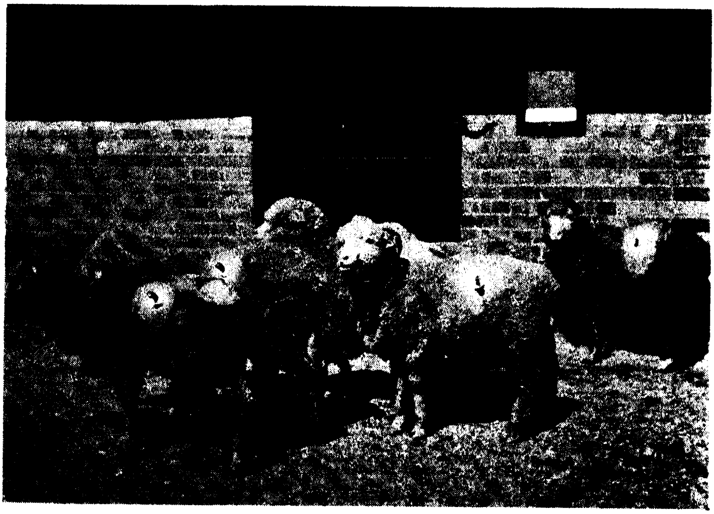

Photo No. 2.—Sheep with "closed" ruminal fistula.

Photo No. 3.—Sheep with closed abomasal fistula.

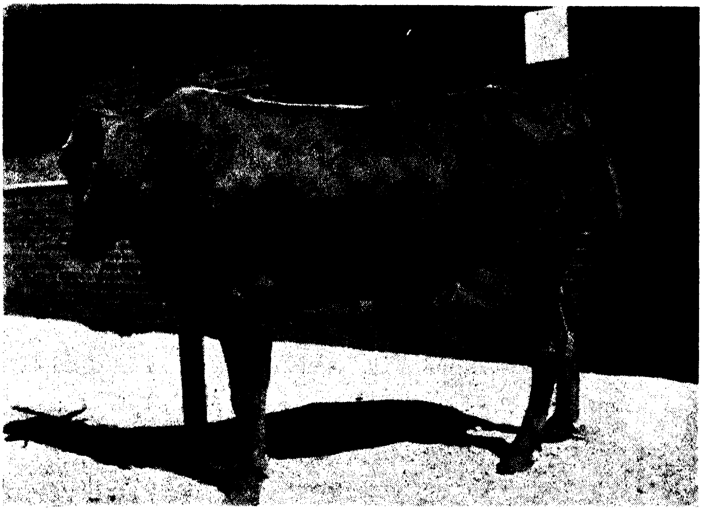

Photo No. 4.—Bovine with open ruminal fistula.

With regard to the insertion of tubes into the intestines, an essentially similar technique is followed except for minor modifications. The intubation of the caecum is generally quite satisfactory, the tube remaining in position very well. In the case of the small intestines, the walls of which are extremely thin in sheep, only small tubes can be accommodated. Even so there is a tendency for such tubes to slough some weeks to some months after their insertion, although the fistula opening in the skin may remain patent much longer. Up to the present, however, our main attention has been devoted to the study of rumen function and hence to the creation of rumen fistulae. All animals are weighed weekly and weight curves plotted. From these data and from the general appearance of the animals, it may be stated without hesitation that the nutritional condition is in no way adversely affected by the presence of the tube in the rumen.

Thus far various aspects of rumen function have been investigated on a small flock of 15 to 18 sheep with ruminal fistulae. It is now intended to describe in some detail the technique followed in the study of each of these phases.

(a) *Kymographic Recording of Ruminal Movement.*

For this purpose the usual balloon and tambour method was at first so modified as to allow easy registration at any selected pressure level. Of the different balloons tried, the ordinary round or oval toy balloon, with a capacity of 60 c.c. at 10 mm. Hg. pressure, was found to yield satisfactory results. One end of a 15 cm. length of thin glass tubing is inserted into the neck of the balloon and this securely tied down on to the tube by a strand of cotton. To allow insertion into the rumen, the deflated balloon is first rolled up so as to form a small scroll which, with the help of a thin probe, is then easily pulled through the fistula tube. By gently blowing into the glass tube attached to the balloon, the latter thus becomes unfolded in the rumen, immediately below the flange of the fistula tube.

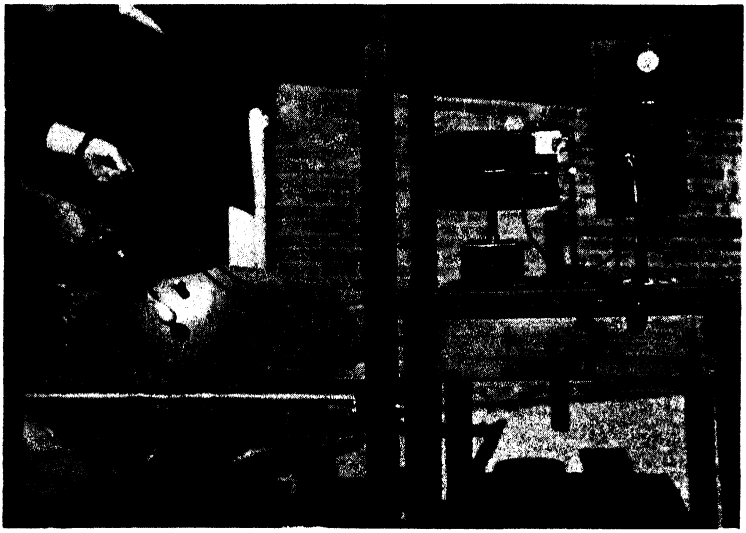

Photo No. 5.—Apparatus for recording ruminal movements. Balloon and tonometer method.

By means of a length of thick-walled rubber tubing, the balloon is connected up with a spring tonometer mounted on a wooden board. By using suitable Y piece connections, this again is brought into communication with a pressure bulb of the type used for inflating the arm band of a sphygmomanometer. From here another length of rubber tubing leads on to a membrane tambour mounted with a suitable writing lever so placed as to record movements on a slow moving clock drum. (See photo No. 5.)

By means of the pressure bulb, the whole system can be inflated to any selected pressure level as indicated on the dial of the tonometer. From experience gained, an initial pressure of 10 mm. Hg. in the balloon and other parts of the recording system was found to be suitable for work on the rumen, and hence this standard adhered to throughout. At this pressure, the whole recording system is extremely sensitive, even minute respiratory changes being clearly registered. Moreover, the actual positive pressures developed in the rumen under varying conditions could be accurately followed. By placing the animal in a specially constructed crush pen, continuous recording of movements, even over the whole day, could be carried out, without interference to the feeding or watering of the animal. Likewise, movements from other parts of the digestive tract such as the abomasum and the caecum could be registered through fistulae in these organs and the use of slightly smaller balloons. As a routine measure all movements are recorded and counted over periods of five minutes at a time, which for the sake of convenience is thus regarded as the unit of recording. In photo No. 6 specimen graphs are shown as obtained from the rumen, abomasum and caecum by this method.

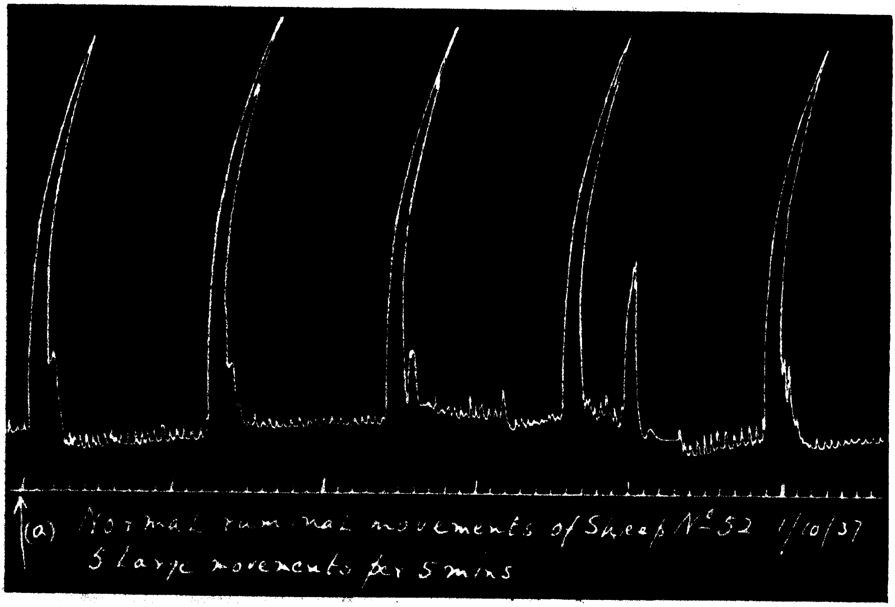

Photo No. 6.—Specimen graphs of rumen.

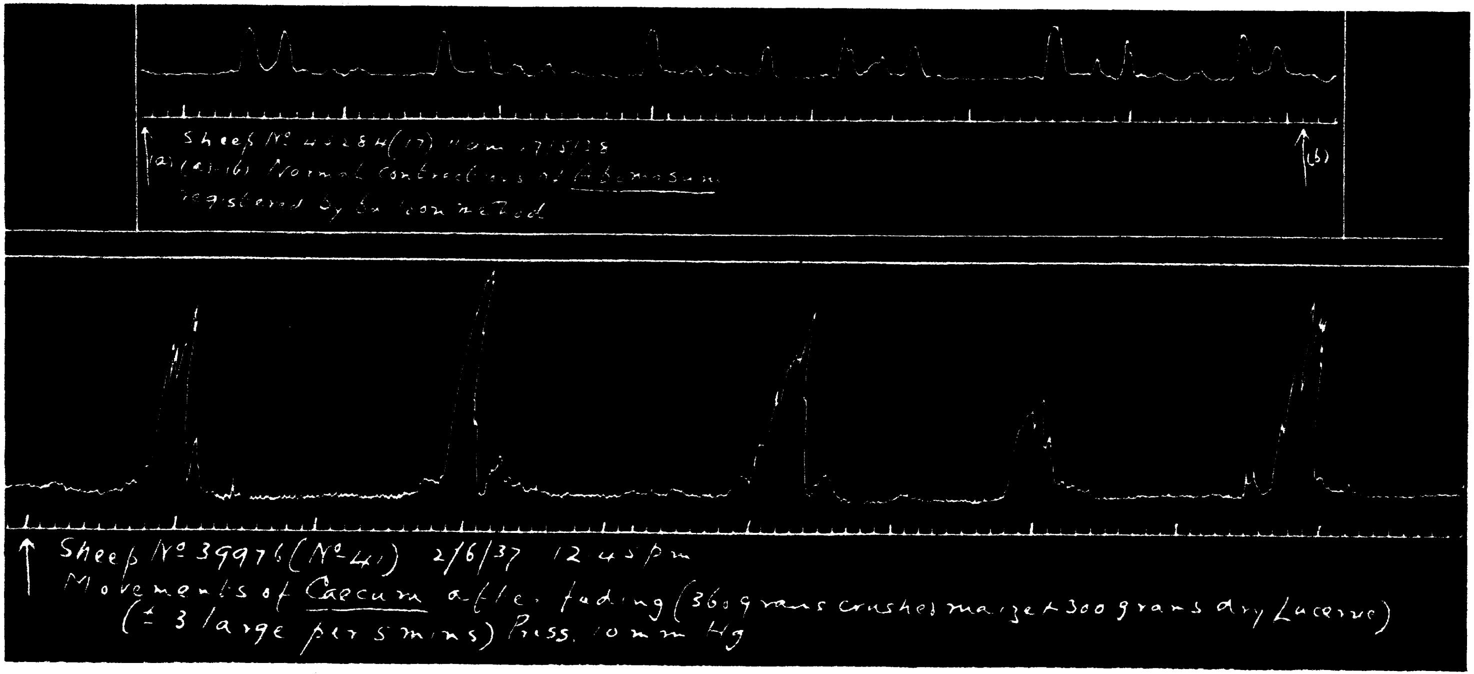

Photo No. 6 (*a*).—Specimen graphs of abomasum (above) and Caecum (below).

By the above balloon method it is, however, very difficult to obtain a clear insight into the maximal and minimal pressures developed with an organ such as the rumen. Moreover such determinations are of importance in a study of the gas pressure associated with fermentation, in which, either normal regurgitation of gas repeatedly takes place through the mouth, or when disturbed may lead to bloating. Consequently it became necessary to improve the above technique so as to allow the accurate recording not only of the individual ruminal contractions but especially also of the limits of pressure associated with any change either in ruminal tonus itself or that due to the accumulation of gas within the organ.

This improvement was achieved by discarding in the first place the use of the balloon in the rumen. By connecting the apparatus directly to the rumen fistula through a short piece of glass tubing, the rumen itself was now made to function as the balloon at the one end of the system. Seeing that the fistula tube in every case is so placed that it is well above the ingesta and water level in the rumen, all changes in pressure are accurately reflected in the rest of the recording apparatus. In no case is there any leakage of gas round about the exterior of the fistula tube when the fistula wound itself is well healed and the flange of the tube drawn up snugly against the abdominal wall.

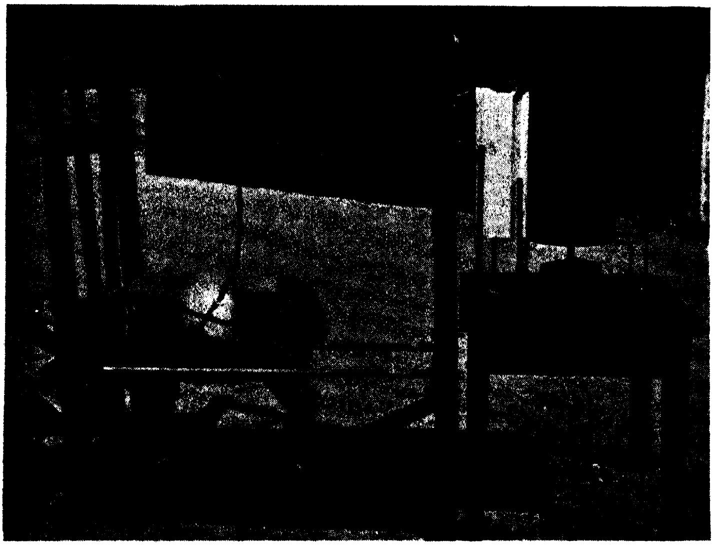

Photo No. 7.—Improved apparatus for recording ruminal movement. Water manometer method.

The second alteration made in the apparatus was to substitute a water manometer, graduated in millimeters (total capacity 220 c.c.) for the spring tonometer. By the use of acidulated water (1 per cent. HCl + methyl red indicator) in the manometer, the possibility of some rumen gas dissolving in it was thereby obviated. Photo No. 7 shows the improved apparatus fitted with the water manometer. By this method it became possible to register in minute detail all the pressure fluctuations, either on the positive or negative side, occurring within the rumen. Also by subsequently changing the pressure in the manometer to the required levels, parallel lines could be drawn on the drum so as to record the limits between which ruminal pressures had actually fluctuated. (See photo No. 8.)

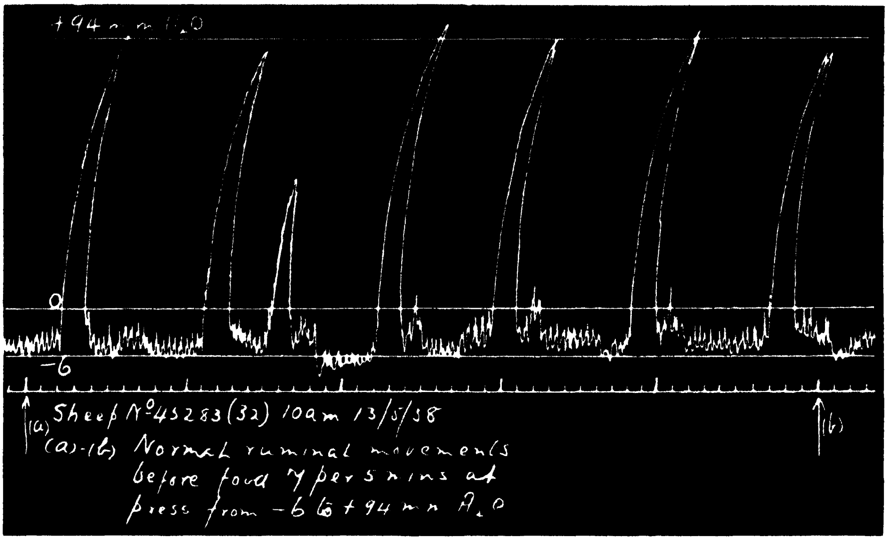

Photo No. 8.—Specimen graph of rumen.

One noticeable feature revealed by such graphs is the frequent occurrence of negative pressures within the rumen of certain animals, a more detailed discussion of which will be given in a subsequent article.

Apart from registering normal ruminal movement, the above method also allows for a detailed recording of the tonus reaction of unstriated muscle of the digestive tract (as represented by the rumen) to a wide variety of factors such as food, drink and action of drugs and other chemical substances.

(*b*) *Sampling of Contents from the Digestive Tract.*

In any study on digestive function, the ease with which portions of the contents can be collected from different levels of the digestive tract, constitutes a very valuable means of investigating the vital processes involved in the disintegration and subsequent absorption and re-excretion of all materials taken in *per os*. Such sampling is readily achieved through fistulae inserted at suitable levels along the digestive tract. Although in the present study, ingesta could repeatedly be drawn off from such regions as the rumen, abomasum, duodenum, ileum and caecum, attention has up till now been focussed mainly on some aspects of the large number of physiological processes occurring within the rumen.

Repeated collection of ingesta from the rumen (photo No. 9) is easily carried out by the insertion of a glass tube through the fistula opening into the depth of the ruminal mass and aspirating by mouth the required amount of ingesta into a splash-head bulb situated in the centre of the sampling tube. Photo No. 10 shows the collection of ingesta from the abomasum.

Photo No. 9.—Collection of ruminal ingesta by aspiration.

Photo No. 10.—Collection of ingesta from abomasum.

From the chemical aspect, ruminal contents have thus far been investigated in respect of moisture content variations, hydrogen ion concentrations and buffer system, the breakdown of different classes of carbohydrates including fibre, and the subsequent elaboration of organic acids both volatile and non-volatile.

Simultaneously with the above investigations, the material from the rumen has been subjected to close microscopic examination both of the bacterial flora and of the infusorial fauna, counts of which were made at repeatedly short intervals. No significant experimental work has, however, been undertaken thus far on the cultivation *in vitro* and the isolation of any organisms.

Significant differences, which will be discussed in subsequent publications, have already been noted in regard to both the chemical and microscopic aspects between animals existing on an adequate level of nutrition as compared to others on a deficient diet.

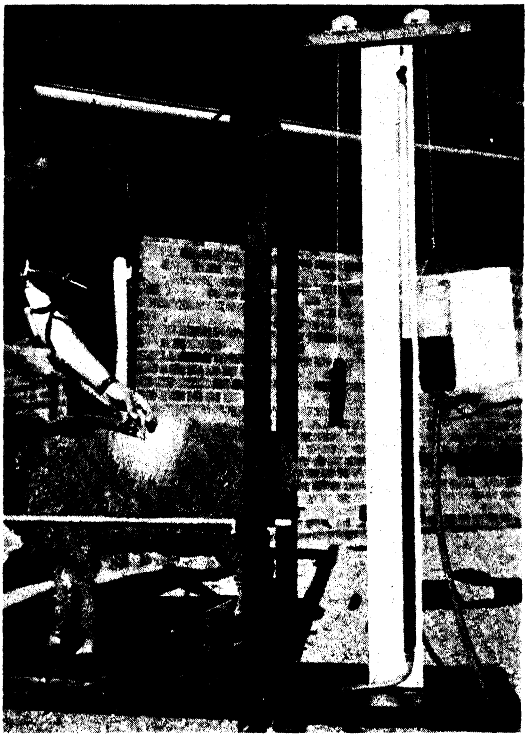

Photo No. 11.—Apparatus for recording total gas volumes from rumen at constant pressure. From side tube sampling of gas for analysis.

(*c*) *Sampling and Recording of Gas from Rumen.*

Seeing that the process of food disintegration within the rumen is associated primarily with fermentation, the continuous recording of the total gas volume liberated, together with an analysis of such gas, furnishes a significant index as to the rate and nature of the fermentation occurring at any given moment, and on a particular type of diet.

A convenient apparatus, devised for the continuous recording of rumen gas, is shown in photo No. 11. It is comprised of a manometer, one limb of which consists of a graduated tube 152 cm. long and 1,130 c.c. capacity, mounted on to an upright wooden stand. This tube is connected by means of a length of stout rubber tubing to a short cylindrical glass reservoir of wide bore, suspended from a pulley and capable of extensive up and down movement. The manometer is filled with the requisite amount of 1 per cent. HCl containing a little methyl red as indicator. The upper end of the graduated tube is connected through a length of rubber tubing directly on to the ruminal fistula in such a manner that any change of pressure within the rumen either depresses or elevates the column of fluid within the graduated tube. By continuous compensatory movements of the cylindrical reservoir, the pressure of the gas can be kept constant at atmospheric pressure. Continuous manometer readings are recorded over periods of 5 minutes at a time, each figure being plotted graphically as it is taken. In this way it is found that a biphasic pressure variation is frequently encountered in the rumen especially after a period of active fermentation, the pressure usually oscillating between a definitely positive and an equally definitely negative phase. This is obviously due to the fact that the pressure exerted by the gas within the rumen is determined not only by the speed and extent of gas formation itself, but largely also by the repeated changes in tonus of the musculature of the rumen. Hence a negative gas pressure is shown whenever the rumen passes into a phase of passive relaxation. By placing the zero line at a suitable level, both phases can be accurately recorded on the graph.

Photo No. 12.—Collection of gas from rumen for chemical analysis.

For the collection and analysis of gas samples, a T-piece with side tube and stopcock is introduced into the system close to the ruminal fistula. Samples of approximately 50 c.c. gas are collected in a small, specially fitted, glass cylinder by displacement of 1 per cent. HCl solution with which the cylinder is filled. (See photo No. 12.)

The gas is subsequently transferred into an evacuated gas pipette, and the analysis carried out in an Orsat-Lunge gas apparatus. The chief components of rumen gas is carbon dioxide which is determined by absorption in KOH solution and methane which is ignited electrically in the presence of oxygen. The percentage methane present is subsequently calculated from the amount of CO_2 formed from its combustion. (See photo No. 13.) Besides these two gases, smaller amounts of hydrogen, sulphuretted hydrogen, and nitric oxide may be encountered within the rumen. Seeing that the process in the forestomachs is essentially of the nature of a controlled acid fermentation, without much evidence of actual putrefactive changes, the formation of SH_2 and other evil-smelling gases is kept down to mere traces under normal conditions of digestion.

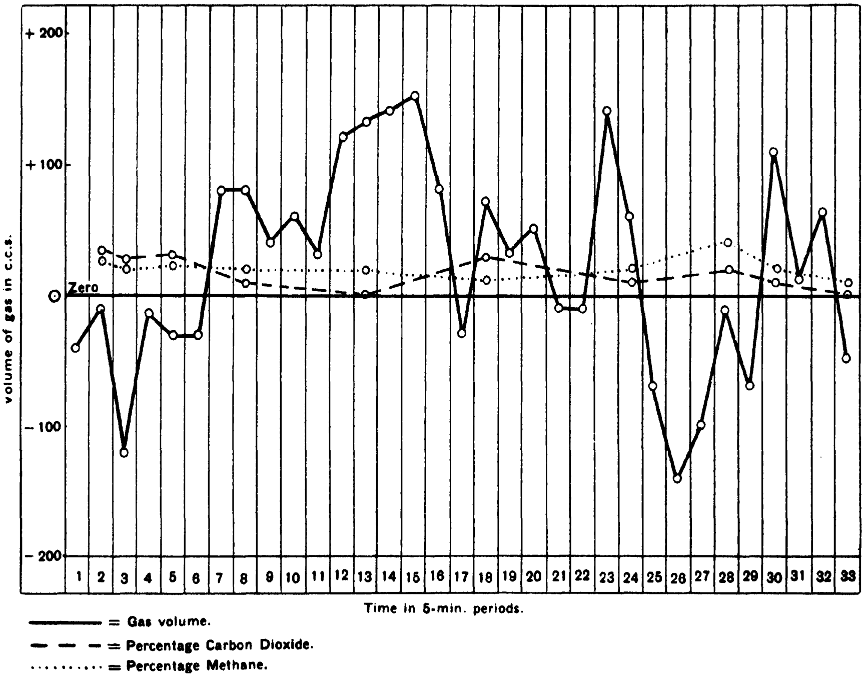

Photo No. 13.—Chart showing fluctuations in normal gas volumes.

(*d*) *Administration of Materials through Fistulae.*

Apart from allowing for the easy collection of contents, fistulae in the digestive tract offer another great advantage in that a wide variety of materials either in solid or in liquid form may be administered through such fistulae directly into any desired part of the tract. Such important factors as rate of passage, nature and speed of disintegration of a substance, disappearance through absorption, effect on digestive tract or animal as a whole, may all then be followed without much of the difficulties usually encountered in studies of this type.

So far, again, attention has been focussed mainly on the rumen. Weighed amounts of substances such as sugar or starch can be dosed through a glass funnel inserted into the fistula tube as shown in photo No. 14. The advantages of this method are that it obviates the necessity of first dissolving the material, frequently in large amounts of water for dosing *per os*, or through a tomach tube. Moreover, it ensures that all the material immediately enters the rumen, thus excluding the possibility of a portion being swallowed through into the abomasum. By these means, such factors as the rate and speed of fermentation of definite quantities of the different foodstuffs can easily be studied in animals existing on different diets, and hence significant data obtained in regard to both the normal and abnormal predigestion of food in ruminants.

Photo No. 14.—Administration of materials through fistula directly into rumen.

Similarly the process of disintegration of coarse materials within the rumen can be followed over an extended period of time. This is achieved by filling small cylindrical bags, made from a very thin natural silk, with samples of the particular foodstuff to be tested, such as dry grain (whole, crushed or shelled) or short lengths of grass or straw (see photo No. 15). By attaching a thin strand of silk to one end, the bag is then inserted through the fistula opening and kept suspended within the rumen by this strand of silk which is fixed to the outer part of the tube after closure of the external opening with its usual rubber cork. (See photo No. 16.) By repeatedly withdrawing the bag from the rumen, and gently washing it in running tap water, the contents of the bag can be examined either grossly or a more detailed microscopic study undertaken of the histological changes taking place within the different plant tissues. Subsequently the same material can be re-introduced into the rumen for a further period of disintegration. This method has also proved of value in comparing the activity of the ruminal flora with that encountered in other levels of the tract such as the caecum when the same materials are suspended through fistulae in the different organs.

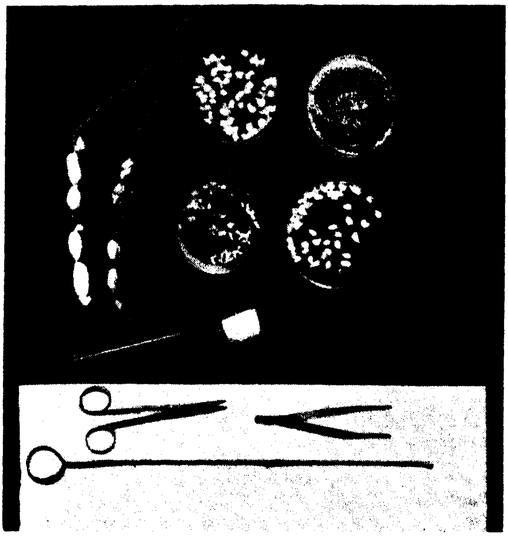

Photo No. 15.—Filling of silk bags with various foodstuffs.

Photo No. 16.—Suspension of silk bags filled with foodstuffs within rumen.

SUMMARY.

1. The importance is stressed of a more intensive study of digestion in ruminant animals, whose food, apart from being disintegrated by enzymes liberated from the digestive tract itself, is first of all subjected to a predigestion, the significance of which is as yet not fully recognised. Through the action of a varied and rich bacterial flora accompanied by large numbers of different species of infusoria, the food mass is exposed to a carefully controlled fermentation occurring within the forestomachs and similar in many respects to that occurring in silage fermentation.

2. In order to study the different stages in the process of digestion, the use of permanent bowel fistulae in experimental animals has been widely resorted to in the past, and an account is given of some of the experimental methods which have been used.

3. An account is given of our experimental technique which involves the use of suitable ebonite fistula tubes and the creation of permanent " closed " fistulae at different levels of the digestive tract of adult Merino sheep.

4. In this study attention has thus far been devoted mainly to some phenomena exhibited within the rumen of fistula sheep, kept on various controlled diets.

5. A method has been devised whereby any pressure change occurring within the rumen could be accurately recorded on slow moving kymographs and the limits of fluctuation in the motility of this organ registered under varying conditions.

6. The technique is described whereby samples of ingesta could repeatedly be collected from the rumen and subjected both to chemical and bacteriological study.

7. By the use of a large water manometer attached to the rumen fistula, continuous volume recording of gas from the rumen could be conducted under constant pressure. Likewise repeated sampling of gas for chemical analysis could simultaneously be undertaken.

8. Methods are described whereby different materials could be administered through the fistula opening directly into the rumen. By enclosing small samples of a variety of foodstuffs within cylindrical bags made from thin natural silk and subsequently suspending these within the food mass of the rumen, the rate at which disintegration took place as also its nature, could be followed microscopically.

ACKNOWLEDGMENT.

In conclusion we wish to express our thanks to Mr. H. B. Carter, University of Sydney, Australia, for the plan of the sheep crush pen used in the foregoing experiments. Likewise, we wish to thank Mr. T. Meyer for the photographs and Mr. C. G. Walker for the drawings and other illustrations, also Messrs. N. K. Gillham and R. J. Briel for valuable assistance in the execution of the experimental work.

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Studies on the Alimentary Tract of Merino Sheep in South Africa. V.—The Motility of the Rumen under Various Conditions.

By J. I. QUIN and J. G. VAN DER WATH, Section of Physiology, Onderstepoort.

INTRODUCTION.

IN a previous article (No. IV) appearing under this series, a description was given of the technique which was followed in studying various aspects of ruminant digestion. As far as the motility of the rumen was concerned, accurate records of this could be obtained from sheep with closed ruminal fistulae. By a direct connexion of the registering apparatus (tambour and water manometer) to the ruminal fistula and without the use of a balloon in the rumen, all pressure changes in this organ could be recorded in detail.

According to recent work conducted by Krzywanek and Quast on sheep with ruminal fistulae, contractions of the rumen are characterised by a series of rhythmically occurring phases in each of which one large contraction precedes two smaller ones. Moreover, it was shown that a close relationship exists between the rumen and the abomasum as far as their motility was concerned, contraction in the latter usually following on that in the former. Wester, in his earlier work on the forestomachs of ruminants, also established the presence of a relationship between the rhythmic movements of different parts of the forestomachs.

Mangold and Klein, and also Czepa and Stygler, on the other hand, were not able to demonstrate any definite and significant type of rhythm for the forestomachs of ruminants.

Due to the fact, therefore, that the characteristic features in ruminal motility are as yet not fully appreciated, it was decided to undertake a series of experiments on fistula sheep with the object of gaining more knowledge in respect of this very important aspect of ruminal physiology.

For the sake of convenience it is proposed to discuss the various experiments concerned with ruminal motility under the following sub-divisions:—

(*a*) The normal motility of the rumen.

(*b*) The influence of food and water (or their absence).

(*c*) The effect of mineral salts.

(*d*) The effect of drugs and poisons.

(*a*) The Normal Motility of the Rumen.

Of the various aspects of ruminal function, that concerned with the movements of the forestomachs is of primary significance seeing that the normal process of food fermentation, including the control of the pH and the eructation of gas, is intimately associated with it. Moreover, as will be shown presently, the rumen is extremely susceptible to a wide variety of factors, each of which may influence the motility in a specific manner and hence also the chemical changes occurring within this organ.

In studying the characteristic features of large numbers of kymographic records obtained from the rumen of clinically healthy and well nourished Merino sheep, it is found that the well-known rhythmic contractions may from time to time undergo significant changes in spite of the animal having been kept under constant conditions.

As a rule it may be accepted that for Merino sheep 5 to 7 ruminal contractions per 5-minute period constitutes the average rate when recorded during the morning and before feeding.

The force and magnitude of the contractions may, however, vary considerably from day to day, although on an average fluctuations between pressures of -10 mm. H_2O during quiescence and $+70$ mm. H_2O pressure at the height of ruminal contraction are commonly encountered. A negative pressure within the rumen, occurring between the periods of contraction, is an interesting feature and one constantly observed in the majority of sheep. Usually it ranges from -5 mm. to -20 mm. H_2O pressure. In a minority of animals, however, the pressure rarely falls below the zero level.

With regard to the rhythm of the movements, a considerable degree of variation may be revealed. Thus it may change from an evenly spaced rhythm in which all the contractions are of approximately the same magnitude to one in which both rate and size of movements become irregular. At times, however, the rhythm described by Krzywanek and Quast may be assumed, although as a rule only for brief periods during which a large movement is followed by two smaller contractions, the first of which takes place soon after the large movement.

With regard to the effect of rumination itself on the movements, the findings of Krzywanek and Quast could be confirmed, in which it was shown that regurgitation of the food mass into the mouth invariably precedes a contraction of the rumen frequently only by a second. Similarly it could be established that contraction of the abomasum was closely related with that occurring in the rumen, the abomasum as a rule showing three waves of contraction immediately after that of the rumen.

Eructation of gas from the mouth usually coincides with a strong ruminal contraction, the frequency of eructation depending upon fermentation activity within the rumen.

Apart from the actual contractions revealed, no significant deviation in the general tonus of the ruminal musculature is noticeable in animals kept under constant conditions.

External stimuli such as repeated pin pricks into the skin over the gluteal region or even fairly intense faradic stimulation of the skin, provoked no significant effect on ruminal movement. The sight of a dog may accelerate ruminal movement considerably, an effect which, however, varies with individual animals.

(*b*) THE INFLUENCE OF FOOD AND WATER ON RUMINAL MOTILITY.

Due to the general significance of ruminal function to nutrition, considerable attention has been devoted in this study to the possible relationship existing between the diet and the motility of the forestomachs. In order to investigate this matter, the procedure adopted throughout was to record ruminal movements firstly before feeding, then during actual feeding and finally at different periods after feeding had been stopped. In all the accompanying graphs, these periods are denoted by arrows, which actually indicate the point at which the conditions were changed. From an analysis of many graphs obtained in this way, it may be stated that in every animal used in the experiment ruminal motility was significantly accelerated from the moment that feeding was commenced until it was stopped again, the average increase ranging from a normal 8 movements per 5 minutes before feeding to 20-25 movements per 5 minutes during feeding, i.e., approximately a threefold increase in the rate of concentration (see graph No. 1). That this acceleration is intimately associated with mastication and deglutition and not with the increased state of filling of the rumen is clearly evident from the subsequent rapid retardation as soon as feeding is stopped again (see graph No. 1). Thus it is frequently found that within 10-15 minutes after feeding the rate of contraction has returned to the normal 8 per 5 minutes, although the individual contractions may still be larger than before feeding. In spite of the fact that the movements are much accelerated during feeding, there is, however, no significant increase in ruminal tonus and intraruminal pressure. On the contrary, there may be a temporary accommodating decrease in tone as shown in graph No. 1, in which the minimal pressure decreases from zero before food to -10 mm. H_2O pressure during actual feeding. This, however, is not consistent for all animals. From these observations, there appears strong evidence that this increased motility of the forestomachs during feeding is associated primarily with vagal reflex action, in which the reflex is initiated probably in the pharynx, during deglutition.

On the other hand, the type of food ingested seems to exert little if any specific influence on ruminal movements, since lucerne (both dry and green), poor veld hay and crushed maize all provoke an equally significant acceleration of ruminal movement during feeding. This, however, is noticed only in hungry animals. Apart from acceleration during actual feeding, some animals, which through repeated handling have become well acquainted with the experimental procedure, show in addition a well-defined psychic response to food, whenever this is shown to it. In such cases ruminal movements are also accelerated, although not to the same extent as during actual feeding. In fistula sheep recently brought under experimental conditions this response may be either absent or not so easily elicited, which indicates that it originates as a type of conditional reflex.

By maintaining animals on any one particular type of food even for prolonged periods at a stretch, the normal rhythm is not disturbed as long as the appetite remains satisfactory. Thus in graph No. 2 the ruminal movements are recorded, both before and after feeding, of a sheep that had been kept on a diet comprised of poor quality wheat chaff only for 8 weeks. As will be noticed, both the rhythm and the size of the excursions were well maintained in spite of the poor diet. When, however, animals are kept on an exclusive grain ration, e.g. crushed maize, for a considerable period, the size of the ruminal excursions become much reduced although the rhythm itself is not significantly altered. In graph No. 3 the ruminal records are shown of two sheep kept on a diet of crushed maize only for several weeks. Where animals are forced to exist for prolonged periods on an unbalanced ration comprised either of crushed maize only or of poor quality straw only, ruminal movements may disappear completely and without warning, this being determined very largely by the idiosyncrasy of individual animals, since cessation of ruminal movement and loss of appetite appear much sooner in some animals than in others.

The dosing of finely ground food, e.g. 200 grams powdered grass hay, directly into the rumen through the fistula opening, causes no change whatever in the ruminal rhythm as shown in graph No. 4, although due to the greater filling of the organ the excursions themselves may be augmented, the maximal pressure increasing from +90 mm. H_2O to +127 mm. H_2O. If, instead of the grass hay, 50 grams of sugar such as glucose is dosed into the rumen, an interesting response may be noted as far as the motility is concerned. In well-nourished animals on a normal diet of lucerne and maize, the usual effect of glucose is that of a rapid rise in intraruminal pressure which may be maintained from 15 to 30 minutes, during which time the contraction resemble an incomplete tetanus which gradually passes of again (see graph No. 5). This increased pressure is due, at least partly, to the rapid increase in the rate of fermentation and the accumulation of extra volumes of gas as clearly shown in volumetric determinations of ruminal gas both before and after the dosing of glucose. The possibility of a direct stimulating effect of the glucose on the ruminal musculature as partly responsible for this effect is, however, not excluded. When, on the other hand, the same amount of glucose is administered to low-conditioned sheep accustomed to a diet of poor quality veld hay only, and in which the animals already show definite signs of body wastage, the opposite response to that described above is very frequently noticed, i.e. there is no sign of an increase in ruminal pressure while the normal contractions themselves may be completely inhibited for periods up to 24 hours or longer. Subsequent determinations of the fermentation rate in the rumen of such animals have moreover shown that the evolution of gas after glucose dosing is either much delayed or even completely kept in abeyance.

Complete starvation of well-nourished animals usually causes no significant effect on the ruminal rhythm for periods ranging from 72-96 hours (3-4 days), although the individual contractions tend to show a progressive decrease in amplitude. After this period a complete cessation of ruminal movement may, however, take place at any time, depending largely on individuality. Through subsequent feeding of such starved animals, ruminal motility usually reappears only after a considerable delay, as the appetite itself may be seriously disturbed after such prolonged fasting.

The Effect of Water.—Whereas food and the process of feeding, exert such well-defined effects on the movements of the rumen, no such response has been noticed for water. Thus neither the actual drinking of water nor the sight of it provokes any significant change in ruminal movement. Moreover, even wide extremes in temperature cause little effect. Thus the dosing of one litre iced water (0° C.) through the ruminal fistula may cause only a slight and transitory acceleration of movement as shown in graph 6. Similarly 1 litre of water at 50° C. provokes little response only, as revealed in graph No. 7. The effects of complete withholding of water for several days is not so easily ascertained, being complicated by the fact that the appetite also soon becomes disturbed, especially when a dry ration only is provided.

(*c*) THE EFFECT OF DIFFERENT MINERAL SALTS ON RUMINAL MOTILITY.

Seeing that it has been shown that sodium salts were effective in causing the closure of the oesophageal groove in cattle (Wester) and that copper sulphate produced a similar effect in sheep, it was decided to ascertain what action, if any, different mineral salts exerted on normal ruminal motility. For this purpose small amounts, usually not more than 2 to 3 c.c. of a 10 per cent. solution of the various salts, were dosed on to the back of the tongue as described by Mönnig and Quin and the response of the rumen to it recorded kymographically, both before and after dosing.

Copper Sulphate.—By dosing copper sulphate solutions to sheep as described above, a striking response is elicited as far as ruminal movement is concerned. This response, however, varies between different animals falling into one of two types: (*a*) In which dosing is immediately followed by a significant acceleration of ruminal movement frequently up to 20 contractions per 5 minutes, closely resembling that noticed during feeding. This effect usually passes off within 5 to 15 minutes, after which the contractions may be somewhat slower and smaller than normal (see graph 8).

(*b*) In this case dosing with $CuSO_4$ solution causes a primary complete inhibition of ruminal movement lasting up to 3 or 4 minutes, after which a secondary stimulation results in the typical acceleration effect as described under (*a*). This is clearly shown in graph No. 9. Moreover it has been found that the response of individual animals to repeated dosing with copper sulphate, when tested out over a period of many months, shows no change whatever, i.e. there is no interchange in response from group (*a*) to group (*b*) or vice versa. That this difference in reaction is closely associated with the closure of the oesophageal groove, is shown by the fact that small glass beads when dosed immediately after the copper sulphate, appear in the faeces of animals showing the (*a*) response, considerably earlier than in those in which the (*b*) response is normally elicited. Thus in the (*a*) group the majority of the beads may be eliminated within 48 hours after dosing, whereas in the other groups small numbers of beads are continually passed out over a period of 7 to 14 days or even longer.

Apart from its effect on ruminal movement, copper sulphate solutions, when dosed on to the tongue, provoke an equally sifinificant acceleration in the movements of the *abomasum* (sometimes after a period of primary inhibition) as shown in graph No. 10, while even the *caecum* is immediately stimulated to a more rapid rate of contraction following the dosing of copper sulphate, as revealed in graph No. 11.

On testing out various other mineral salts, it has been ascertained that silver nitrate solutions provoke a response identical to that of copper sulphate, as revealed in graph No. 12. Apart from the mineral salts, nicotine sulphate also causes a similar reaction in ruminal motility, as shown in graph No. 13. Of all the other mineral salts, such as magnesium sulphate, sodium sulphate, sodium chloride, ferrous sulphate and aluminium potassium sulphate (alum), when dosed in the above manner, none of these provoke any significant change in normal ruminal motility (see graphs Nos. 13, 14, 15, 16, 17). Thus it appears to be clearly established that only a limited few substances such as copper, silver and nicotine are capable of eliciting the vagal reflex in sheep when swallowed.

In addition to the above mineral salts, the effect of potassium cyanide on ruminal movement has also been ascertained. By dosing amounts ranging from 150 to 200 mgs. potassium cyanide dissolved in a few cubic centimeters of water directly into the rumen, an extremely rapid decrease in the rhythm and size of the contractions may be noticed which soon culminates in complete paralysis of the organ, while the respirations become much deeper and dyspnoeic in nature. See graph No. 18. Depending upon the dose and the general severity of the reaction, a return of the ruminal movements may be expected usually in 10 to 40 minutes after dosing, the size of the contractions gradually increasing with recovery. Equal doses of KCN may, however, provoke distinctly different effects on animals kept on different diets? Thus it has been found that a dose of 180 mgs. KCN, when dosed to a sheep on a diet of lucerne hay only, causes an effect much milder than that noticeable in animals on a mixed diet of lucerne and crushed maize. Due to the slightly more acid reaction of the ruminal contents on the latter diet, it would appear that the more severe reaction is associated with a correspondingly more rapid formation, and hence a higher concentration of easily diffusible hydrocyanic acid gas from the KCN within the rumen.

(*d*) THE INFLUENCE OF DRUGS ON RUMINAL MOVEMENT.

As certain drugs are reputed to exert a powerful effect on plain muscle, experiments were undertaken in which several well-known drugs in addition to various other substances were tested out for their action on ruminal motility. Of these, three vagal stimulants, viz. acetyl-choline, Lentin (Merck), and Esmodil (Bayer) may be regarded as comprising one group. Acetyl-choline, now generally accepted to be constantly liberated under parasympathetic stimulation was tested out on ruminal fistula sheep either alone or in combination with eserine sulphate. It was found that acetyl-choline in doses of 10 mgs. and more intravenously (dilution 1 : 1,000) caused an immediate and complete paralysis of ruminal movement, similar in all respects to the action of HCN on the rumen.

The effect of the acetyl-choline is, however, of very short duration, since rapid recovery of ruminal movement may be expected within 10 minutes after administration (graph No. 19).

Esmodil (Bayer) in a dose of 3 mgs. (dilution 3 : 1,000) intramuscularly was found to cause a progressive acceleration of ruminal movements accompanied by a rise in the general tone of the rumen. Although somewhat similar to Lentin (Merck) in its action, Esmodil appears to exert a milder effect on the plain muscle of the rumen (graph No. 20).

Lentin (Merck) in a dose of 1 mg. (1 : 1,000) injected subcutaneously, causes a practically instantaneous change both in ruminal tone and in rhythm. The tone which rises to well above the normal level may remain in a spastic state for a period of one hour or longer during which the normal movements are replaced by a series of rapidly recurring, shallow, wave-like contractions resembling those noticeable during a state of incomplete tetanus (graph No. 21). That these cramp-like peristaltic contractions are present at least also in the large bowel is proved by the fact that repeated purgation may set in within 45-60 minutes after injection. At the same time glandular activity is markedly stimulated as evidenced by the profuse and continued salivation and lachrymation. However, all the effects are usually passed off within 2½ to 3 hours, leaving the rumen with its normal rhythm of contraction.

Apart from the above-mentioned three substances, various other drugs were tested out on the rumen. Of these, pilocarpine hydrochloride (graph No. 22) and arecoline hydrobromide (graph No. 23) in doses of 25-40 mgs. per sheep intramuscularly provoked a response resembling that caused by Lentin, except that in no instance has purgation been noticed in sheep following the injection either of arecoline or pilocarpine.

The results with eserine sulphate have been disappointing in that even large doses of 10-15 mgs. intramuscularly failed to provoke any definite response from the rumen except augmentation of the ruminal movements (graph No. 24).

Veratrine hydrochloride, in doses of 5 mgs. subcutaneously, produced intense local irritation and a definite slowing down of the ruminal movements (graph No. 25).

Adrenalin (1:1,000) in doses of 2 c.c. subcutaneously, failed to provoke any change in the normal rhythm or tonus of the rumen (graph No. 26).

A poisonous principle isolated from the plant *Lippia rehmanni* (Verbenaceae), termed *icterogenin*, was found to produce a complete and prolonged inhibition of ruminal movement associated with loss of appetite when given *per os* in an amount of 4 grams (graph No. 27). This substance, which has been shown to be in the nature of a resinic acid, likewise causes inhibition in the bile excretory mechanism of the liver associated with a progressive severe type of regurgitative jaundice.

DISCUSSIONS AND CONCLUSIONS.

Experiments conducted on sheep with ruminal fistula have shown that normal ruminal movement may undergo very considerable variations from any rhythm that may be temporarily established. In this respect it is essentially the strength of the individual contractions as well as the spacing of the movements which may undergo a sudden change rather than the rate and the tonus of the rumen both of which remain far more constant. An interesting feature is the extensive degree of relaxation undergone by the ruminal musculature between the actual contractions. During these periods of rest the intra-ruminal pressure usually falls well below zero, values of -5 to -15 mm. H_2O pressure being frequently encountered. At the height of contraction the intra-ruminal pressure may rise from $+20$ to $+129$ mm. H_2O, with an average of ± 70 mm.

Feeding in every instance causes a very significant acceleration in the movement of the rumen, this being due in all probability to vagus reflexes initiated in the pharynx and oesophagus, and closely associated with mastication and deglutition. In some animals the mere sight of food may initiate reflex acceleration of ruminal movement. This effect is, however, neither as constant nor as strong as that noticeable during actual feeding. The type of food exerts little, if any, effect on the ruminal rhythm, although contractions tend to become small when animals are kept on a ration comprised exclusively of concentrates.

Complete starvation as a rule causes no definite change in the rate of ruminal movement up to the third or even the fourth day, although the excursions may become progressively smaller. After this period all movements may, however, disappear completely. Following renewed feeding after such prolonged starvation, a definite delay in the return of movements and also of the appetite is frequently noticeable.

The influence of water on the rumen is much less specific seeing that neither the sight of water nor actual drinking causes any appreciable change in the motility of the rumen. Moreover. wide extremes of temperature ranging from 0° C. to 50° C. following the dosing of 1 litre of either iced or warm water cause very little change in normal motility.

With regard to the influence of various mineral salts, it has been established that small doses of a copper sulphate solution, as well as similar solutions of silver nitrate and nicotine sulphate, when dosed on to the back of the tongue all cause very profound changes in ruminal rhythm, due again probably to a vagal reflex. All other mineral salts tested out so far have failed to provoke this charecteristic response from the rumen.

Potassium cyande in small doses *per os* causes a rapid though transitory paralysis of all ruminal movements.

In addition to the mineral salts, various other substances have also been tested for their action on the rumen. Of these, Lentin (Merck) and Esmodil (Bayer) have been found to cause a well-marked stimulation of ruminal movement, while acetyl-choline causes an acute though transitory paralysis. Pilocarpine and arecoline also cause significant stimulation of ruminal movement. Adrenaline subcutaneously in doses of 2 c.c. (1:1,000) was found to exert little, if any, change in ruminal motility.

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Section IV.

Chemical Pathology.

ROETS, G. C. S. ... ... Coproporphyrin excretion in East Coast fever of cattle 385

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Coproporphyrin Excretion in East Coast Fever of Cattle.

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THE object of this note is to put on record observations concerning the presence of porphyrin in the urine and faeces of bovines experimentally infected with East Coast fever, at Onderstepoort. These observations are noteworthy in that they constitute additional evidence of the excretion of porphyrins in pathological conditions. Moreover they contribute as far as can be ascertained the first definite evidence of the excretion of these substances in a protozoal disease like East Coast fever.

The series III porphyrins, which are regarded as the normal "physiological" type, occur in certain pathological conditions including cirrhosis of the liver (Dobriner, 1936), hepatic tumours (Dobriner, 1936), lead poisoning [Grotepass (1932), Mertens (1937), Fischer and Deusberg (1932), and after the administration of salvarsan (Schreus, 1935) and sulphonilamide (Rimington, 1938). In congenital porphyrinuria, series III, porphyrins are found to be excreted simultaneously with excessive amounts of series I [Fischer and Libowitzky (1936), Fischer and Hofman (1937), Rimington (1937), Rimington and Roets (1937)]. In idiopathic porphyrinuria series III has been demonstrated by Waldenström (1935) and Mertens (1936), whilst Dobriner (1936) detected the presence of the I and III series in the faeces of one case.

In the East Coast fever cases investigated this duality of the coproporphyrins was found. The total coproporphyrin extracted from faeces having a porphyrin content of 94-104 γ per 100 gm. fresh faeces, was esterified and the methyl esters separated by dissolving the Copro III ester in cold methyl alcohol. In one animal the ratio of Copro I : Copro III was 1 : 1·15, whilst in another only a trace of the I series was present.*

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* Determinations were made by the methods described by Rimington, Roets and Fourie in a paper "Quantitative Studies upon Porphyrin Excretion in Bovine Congenital Porphyrinuria", (Onderstepoort Jnl. of Vet. Sci. Vol. 10, No. 2, p.p. 421-430, 1938).

Working with a disease like East Coast fever the danger of spread of the disease must constantly be guarded against. The experimental animals had to be closely confined and the conditions were such that it was impossible to collect and examine their urine daily; consequently what urine it was possible to collect was worked up and the extracted porphyrins accumulated. In such urine the ratio of Copro I : Copro III was found to be 1 : ·31.

The copro I ester, see Fig. 1, has a melting point of 240-244°. The M.P. of a mixture of this ester and coproporphyrin I ester, of M.P. 240-246° which was obtained from the faeces of congenital porphyrinuric animals was 240-246°. Therefore there was no depression. The spectrum, solubility and acid number are like that of copro-porphyrin.

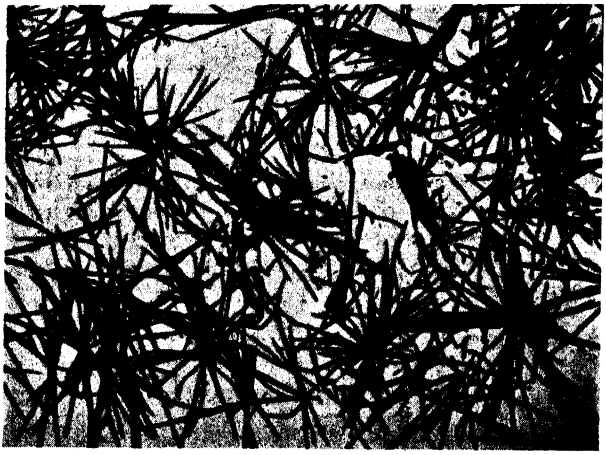

Fig. 1.—Copro I ester. Magn. 300×.

The Copro III ester crystallised in small clusters of fine needles, see Fig. 2, on allowing a solution to evaporate spontaneously in a tube either from methyl alcohol or ether (compare Rimington, 1938). The melting point of the combined ester from the urine and faeces was found to be 136-170°, remelt 160°. It has the coproporphyrin spectrum acid number and solubility.

It might be mentioned here that 14-25 γ coproporphyrin was detectable in 100 gm. samples of fresh faeces before the animals were infected, which agrees with Dobriner's findings, namely that coproporphyrin is normally excreted in small amounts in faeces (Dobriner, 1937). Further investigations are being continued with the object of obtaining data which may contribute towards a clearer understanding of the relationship existing between the natural porphyrins and the process of haemopoeiesis in a disease such as East Coast fever.

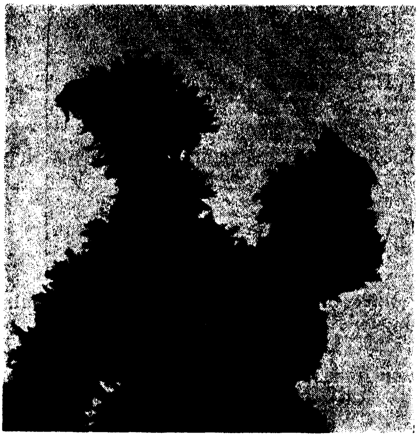

Fig. 2.—Copro III ester. Magn. 300×.

SUMMARY.

1. Coproporphyrin I and Coproporphyrin III were isolated from the faeces and urine of cattle suffering from East Coast fever.

2. The ratio of the I to the III series in the faeces of one animal was 1 : 1·15, whilst in that of another only traces of the I series could be detected.

3. In an accumulated sample of coproporphyrins extracted from the urine of East Coast fever cattle the ratio of the I to the III series was found to be 1 : ·31.

ACKNOWLEDGMENT.

The author wishes to thank Mr. W. O. Neitz for kindly putting material at his disposal.

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Section V.

Nutrition.

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Plant Proteins. IV.—The Biological Values of Soyabeans, Linseedmeal and Soyabeans Supplemented by Cystine.

By D. B. SMUTS and J. S. C. MARAIS, Section of Nutrition, Onderstepoort.

THE present investigation constitutes a continuation of our studies on the nutritive value of our most commonly utilized plant proteins. Linseedmeal is a popular feed for nearly every type of farm animal and consequently is extensively used in this country. Soyabeans are less popular, but due to the fact that they may be grown fairly successfully under local conditions, their nutritional value in comparison with other plant protein becomes a practical necessity.

Morris and Wright (1933), in a comparison of the proteins of beans, linseed and meatmeal for milk production came to the conclusion that beans and meatmeal are better sources of protein for milk production than linseedmeal. The biological values calculated according to the formula of Perkins ranked in the following order: Beanmeal 59, meatmeal 55, and linseedmeal 46. Crichton (1932), in a fattening test of two groups of Aberdeen Angus steers on bran and linseed cake, states that the bran ration gave better gains than the linseed ration. Mitchell (1931), determined the nutritive value of linseedmeal and cottonseedmeal for growing pigs and found that, while linseedmeal was slightly more digestible than cottonseedmeal, there was no actual difference in their respective biological values; the values being 61 for linseedmeal and 63 for cottonseedmeal. Kenneth, Morrison and Maynard (1935), employing their synthetic rations developed for sheep, found in a comparison of the biological values of soyabeans, corngluten and linseedmeal, that the latter was inferior in protein to soyabeans, the respective biological values being 72·8 for soyabeans and 67·7 for linseedmeal.

Csonka (1934), from chemical analyses of various varieties of soyabeans, does not support the general conception of a quantitative deficiency of cystine in soyabeans but admits that some varieties may be deficient in this amino acid. Hayward, Steenbock and Bohstead (1936) found, with rats, that both the Illini and Herman varieties of soyabeans were inefficient sources of protein. The addition of 0·3 per cent. l-Cystine or the application of heat practically doubled the nutritive value of the protein. The commercial preparation of soyabeanmeal apparently influenced its protein constitution markedly. Low temperature preparations at 105° C. for 2 hours or cooked at 82° C. for 90 minutes left the protein unchanged and similar in nutritive value to the raw product. Cooking at 105° C. to 121° C. for 90 minutes changed the protein to such an extent that it became double the nutritive value of the raw meal. Plimmer and Rosedale (1934), in an extensive investigation of the relative values of different proteins, as determined by the growth method on chickens, found that soyabeans were superior in protein to linseedmeal but inferior as compared to fishmeal and meatmeal. Schmidt (1934), in a determination of the nitrogen retention of yeast, soyabeans and peanutmeal in pigs, found that the nitrogen retention per head was 13·69 gm. for yeast, 13·22 gm. for peanutmeal, and 13·22 gm. for soyabeans.

In accordance with the general belief that soyabeans are deficient in cystine, this amino acid was incorporated in a ration of soyabeans and the biological value determined. Until quite recently cystine was thought to be an indispensable amino acid. Rose (1937), however claims that this is not the case and that methionine is the indispensable sulphur amino acid, while cystine only acts as a stimulant to growth when methionine is present in sub-optimal quantities. Weichselbaum (1935), on the other hand, finds that on a cystine deficient ration rats develop a specific syndrome, which can be prevented by both cystine and methionine, but that only the former amino acid can rectify conditions once symptoms have appeared.

EXPERIMENTAL.

The biological value of each protein, except for linseedmeal, was determined on six individual rates. Due to the scarcity of rats an unavoidable change in the general routine of our metabolism work was brought about. Instead of running the usual nitrogen low and protein periods on six rats and then discarding them, the rats under the present conditions were employed for two successive periods of protein feeding and one nitrogen low period to serve for both protein periods. In our experience this deviation does not constitute a serious source of error, since the endogenous nitrogen of rats over a period of a month does not vary to any appreciable extent, especially if they are kept on a standard ration of 10 per cent. protein before the endogenous nitrogen is determined. The rations were compounded to approximate 9 per cent. of protein and to contain all the other essential ingredients for normal growth. The protein feeds investigated were put through a mill and ground into a fine state in order to facilitate homogeneous mixing. The cystine incorporated in the rations was prepared from wool. The percentage composition of the rations are given in Table 1.

TABLE 1.

Composition of Rations on Percentage Basis.

Ingredients.	N-low Ration.	Soyabean Ration.	Linseed Ration.	Soyabean and Cystine Ration.
Soyabeans	—	35·2	—	34·7
Linseed meal	—	—	26·1	—
Cystine	—	—	—	·20
Ether Ext. Egg White	3·8	—	—	—
Sucrose	10·0	10·0	10·0	10·0
Yeast Ext.(¹)	10·0	10·0	10·0	10·0
Butterfat	8·0	8·0	8·0	8·0
Codliveroil	2·0	2·0	2·0	2·0
Salt Mixture(²)	4·5	4·5	4·5	4·5
Agar	2·0	2·0	2·0	2·0
Starch	58·7	27·3	38·4	29·6
NaCl	1·0	1·0	1·0	1·0
TOTAL	100·0	100·0	100·0	100·0
Per cent. Nitrogen	·650	1·51	1·50	1·46

(¹) Yeast extract was prepared according to the method of Itter, S., Orent, E. R., and McCollum, E. V., 1935. *J. Biol. Chem.*, Vol. 108, No. 2, pp. 571-577.

(²) A modified Osborne and Mendel salt mixture described by Hawk, P. B., and Osler, B. L., 1931. *Science*, Vol. 74, p. 369.

The nitrogen metabolism data as well as the calculation of the biological values are presented in Table 2.

From these figures it will be seen that the protein of raw soyabeans is of an inferior quality. In fact, it exhibits the lowest biological value of all the plant proteins so far investigated. The individual biological values vary from 50 to 62 with an average value of 55. The apparent and true digestibilities are lower than those of other plant proteins, except lucerne, and average 62 and 81 per cent. respectively. It is fairly obvious that the low digestibility is not the primary cause of its poor nutritive value, since when soyabeans are supplemented by ·20 per cent. cystine the apparent and true digestibility remain practically unaltered, whereas the biological value of the supplemented soyabean protein is increased significantly. Such a decided increase in the biological value definitely signifies a supplementary effect of cystine on soyabeans, and can only be interpreted on the grounds that a deficiency of this amino acid in the protein molecule of soyabeans seriously impairs and diminishes the utilization of its nitrogen. The result is somewhat opposed to the new theory of the dispensability of cystine as recently postulated by Rose (1937). According to Rose methionine may be present in suboptimal quantities in soyabeans and the addition of 0·2 per cent. cystine simply as a stimulant to growth. This argument, it seems, does not fully explain the metabolism data presented in this paper. If cysine is dispensable one would hardly expect that its inclusion in such small small quantities would increase the utilization of soyabean nitrogen for the composite needs of maintenance and growth as is clearly demonstrated by our data. In fact one would expect the addition of an amino acid dispensable for tissue synthesis to decrease the biological value instead of enhancing it, since such an amino acid will be added to the fraction of incomplete assortment of amino acids and deaminized with no actual use for the body. The indispensability of cystine in soyabeans which might contain methionine in suboptimal quantities is further supported by data obtained from paired feeding tests, to be published shortly, in which soyabeans supplemented by cystine are superior to soyabeans alone.

From the biological value of linseedmeal as calculated in Table 2 it is apparent that its almost universal use in stock feeding is not only based on its popularity and palatability but to a large degree on the high quality of its protein. The individual biological values vary from 74 to 82, with an average of 78. Its apparent and true digestibility, as shown in Table 3, are of the same magnitude as those of most other plant proteins; the respective values being 75 and 98 per cent. This would indicate that the higher biological value of linseedmeal in comparison with peanutmeal, sesamemeal, and coprameal as determined in a previous paper [Smuts and Malan (1937)] is due to a better constituted protein molecule in respect to the indispensable amino acids.

TABLE 3.

Summary of Apparent and True Digestibilities.

Rat No.	Soyabean.		Rat No.	Soyabean and Cystine.		Linseed.	
	Apparent.	True.		Apparent.	True.	Apparent.	True.
1	64	81	7	57	76	75	94
2	60	79	8	64	86	74	96
3	62	81	9	67	93	75	100
4	69	88	10	69	88	76	94
5	60	79	11	57	79	75	97
6	59	78	12	66	85	73	91
Average.	62	81		63	84	75	95

SUMMARY AND CONCLUSIONS.

By means of the nitrogen balance-sheet method a biological value of 55 for soyabeans and 78 for linseedmeal at approximately 8 per cent. level has been obtained. Utilizing the same method and substituting an equivalent amount of soyabean nitrogen by ·20 per cent. cystine it was found that cystine supplemented soyabean protein effectively and increased the utilization of its nitrogen by 19 per cent.

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Plant Proteins. V.—The Biological Value of Lucerne and Lucerne Supplemented by Cystine in Sheep.

By D. B. SMUTS and J. S. C. MARAIS, Section of Nutrition, Onderstepoort.

In experiments with rats (1937) it was shown that lucerne protein fed at approximately 8 per cent. level, gave the lowest biological value of all the plant proteins so far investigated. This defective utilization of the lucerne protein by rats was then definitely shown to be caused by a deficiency of the amino acid cystine, which, when added to lucerne in minimal quantities, enhanced its biological value considerably. In view of these findings, together with the controversial opinions in the literature as to the rôle of cystine and sulphur-compounds in the nutrition of sheep, it was regarded as essential to attack the question from a somewhat different angle and to ascertain whether the addition of cystine to lucerne has any effect on the biological utilization of its nitrogen by sheep.

Until quite recently the indispensability of cystine in the nutrition of the rat has been recognized universally. Rose (1937) now queries the indispensability of this amino acid and states that it only acts as a stimulant to growth when methionine is present in suboptimal quantities, and that the latter is in reality the indispensable component. However, a number of workers [Haag, T. R. (1933), Mitchell, H. H. (1930), Smuts, D. B. (1937), Schrewsbury and Bratzler (1933), Weichselbaum (1935), and Scoz (1932)] have shown that the addition of cystine enhances the growth-promoting properties of a protein feed deficient in this amino acid, and that it is necessary for normal nutrition. Furthermore, it has been shown that the addition of minute quantities of cystine to a cystine deficient ration markedly increases the utilization of the absorbed nitrogen of such a ration. These observations, it appears, can only be explained by assuming that the added cystine supplemented the existing amino acid complex in the protein molecule, thereby creating an assortment of amino acids more suitable for the selective needs of tissue synthesis. The cystine inclusion therefore avoids that wastage of amino acid nitrogen which, in its absence, would have been incompletely utilized and must consequently be regarded as essential in the process of tissue construction. On the other hand, Jackson and Block (1932) have shown that on a cystine-methionine free diet d-methionine and formyl *l* methionine can stimulate growth in rats. Weichselbaum (1935), on a Sherman-Merril cystine deficient diet, found that rats develop a syndrome which can be prevented by either methionine or cystine and that cystine alone could overcome symptoms when they have definitely appeared.

Whatever explanation may be forthcoming, the preponderance of evidence at present favours the indispensability of cystine in the nutrition of the growing rat. It further appears that apart from methionine no other known amino acid can replace cystine in a ration or that the latter can be synthesized by the rat from simpler organic or inorganic sulphur-containing compounds. Rose and Huddlestin (1926) and Lewis and Lewis (1926) found that taurine, a derivative of cystine, could not supplement a cystine deficient diet for maintenance and growth. Westerman and Rose (1927) obtained no evidence that thioglycollic acid and dithio-dipropionic-acid could be utilized for synthesis of cystine by the rat.

As far as synthesis of cystine from inorganic sulphur is concerned only negative results have been obtained. Daniels and Rich (1918) found inorganic sulphates incapable of covering a cystine deficiency either for growth or maintenance. Geiling (1917) obtained no change in the declining weight of mice on a cystine deficient diet when flowers of sulphur were added to it.

While the indispensability of cystine in the absence of sufficient methionine holds true for rats and other omnivores, it seems quite possible that ruminants with their complicated and differently constructed alimentary tract may react otherwise. In fact, the synthesis of vitamin B by the rumen flora of cattle, as postulated by Bechdell, Honeywell, Durcher and Knutser (1928), strongly indicates that there are distinct differences in the digestive activities of these two types of animals. Reasoning on more or less analogous grounds, Rimington and Bekker (1932) suggested that sheep may be able to synthesize cystine through a symbiotic action of the intestinal flora. Fraser and Fraser-Roberts (1932), in trying to explain the high percentage of cystine in wool suggest that the wool follicles themselves may perform this special function of transformation into cystine. These theories, although probable, can only be solved one way or the other if such factors as the cystine content of the ration, percentage utilization of cystine for maintenance, growth, and wool production, and the cystine content of the fleece. are determined. In the absence of the latter information, it appears but naturel to accept Woodman and Evans' (1932) explanation that there is enough cystine in pasture to satisfy the needs of growth and wool production in sheep.

In our work on the determination of the biological value of lucerne for sheep, it seemed as if we could obtain some information on the cystine problem by supplementing lucerne with cystine and determining its biological value. If cystine is indispensable for sheep and the cystine and methionine content of lucerne too low for maintenance, then one would expect an enhanced biological value with the addition of cystine if no synthesis of this amino acid is accomplished.

EXPERIMENTAL.

Four mature wether merinos were used in this experiment. They were transferred from a standard protein ration to a nitrogen-low ration for 15 days, after which period they were put in metabolism cages. The collection periods lasted ten days, and the preliminary feeding periods of the protein rations also ten days. The feed for each experimental period was weighed out at the start, and from it the daily food requirements were weighed out and placed in paper bags. Representative samples were taken and analysed in triplicate. Sheep were fed once daily and any unconsumed feed of the day collected, air-dried and stored.

Urine was collected daily in 2 per cent. HCl and the urine, together with the washings made up to a known volume, from which a 10 per cent. aliquot was taken and stored. The daily collection of faeces was air-dried, weighed, aliquoted and stored in air-tight containers until analysed. The composition of the rations is given in Table 1. By lucerne hay is meant sun-dried hay which was chopped in convenient lengths to prevent spilling.

TABLE 1.

Composition of Rations.

	A.	B.	C.
Lucerne hay	58·0	56·0	—
Cystine[1]	—	·20	—
Codliveroil[1]	2·0	2·0	2·0
Salt	2·0	2·0	2·0
Bone ash[2]	3·0	3·0	3·0
Dex starch	45·0	36·8	73·0
Agar	—	—	20·0
TOTAL	100·0	100·0	100·0

[1] Cystine was prepared from wool and contains ·114 per cent. N in comparison with the theoretical value of ·116 per cent. N. The latter figure was utilised.

[2] Bone ash contains 42·5 per cent. Ca and 17·04 per cent. P.

EXPERIMENTAL RESULTS.

The results of the metabolism experiments, together with the calculation of the biological values, are given in Table 2. As will be seen, the order in which the different periods appear was as follows: Nitrogen free period, 8 per cent. lucerne protein, 8 per cent. lucerne protein supplemented by cystine, followed by a nitrogen free period. The results of the first nitrogen free period were utilized in the calculation of the biological value of the 8 per cent. lucerne protein ration. The same procedure was followed in calculating the biological value of lucerne supplemented by cystine by using the figures obtained in the second nitrogen free period. In graph 1 the metabolic faecal nitrogen for the two nitrogen free periods is plotted against the corresponding quantities of dry matter intake. These points, except for one, very nearly assume a straight line showing, what is generally accepted, that there is a strong positive correlation between dry matter intake and metabolic faecal nitrogen elimination.

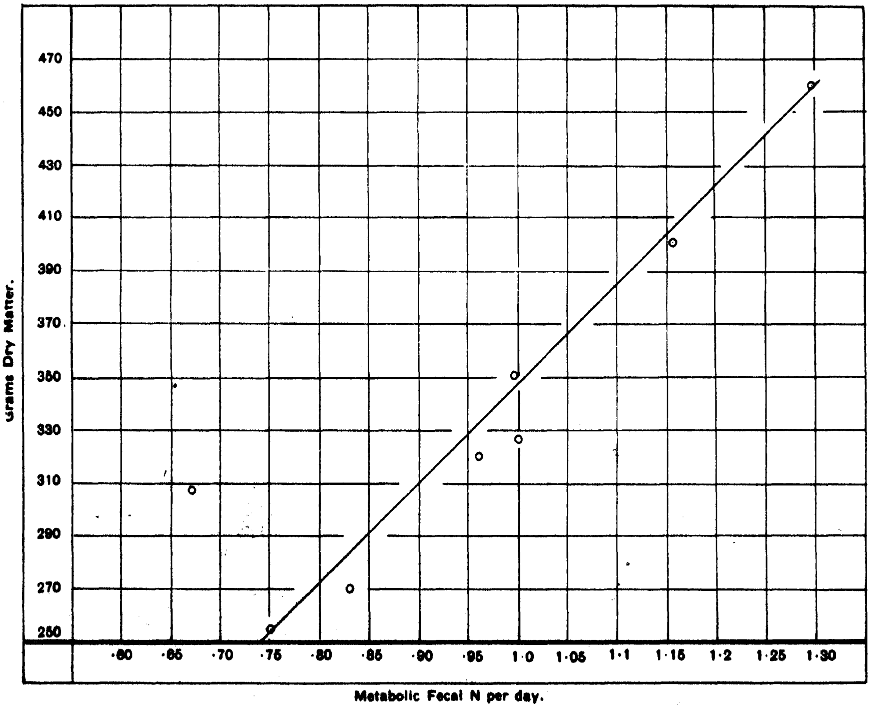
TABLE 2.

The Apparent and True Digestibility of Lucerne and Lucerne Supplemented by Cystine.

Animal No.	LUCERNE.		LUCERNE CYSTINE.	
	Apparent.	True.	Apparent.	True.
1	71	90	57	76
2	77	97	59	79
3	74	88	64	82
5	79	87	65	82
	75	91	61	80

GRAPH 1.

Relation between Metabolic Fecal N and Dry Matter Consumed.



The average biological value obtained for lucerne protein at an 8 per cent. level is 60. Sheep 1 and 2 in both periods of protein feeding gave higher biological values than sheep 3 and 5. This fact is not accounted for by a difference in digestibility, but is in all probability due to a better utilization of the absorbed nitrogen, since there is not much difference in the magnitude of endogenous nitrogen excretion between these sheep per Kg. body weight. The fact that this difference in biological values is so consistent in the two different periods show that it must be due to some inherent difference in the physiological abilities of these sheep to utilize the absorbed nitrogen better than their two companions.

The value 60 obtained by us corresponds well with the value obtained for this protein with rats and also agrees very closely with the value reported by Satola (1930) for sheep. Satola's values vary from 53 to 60, with an average of 56. The value reported by Turk, Morrison and Maynard (1934) are much higher and average 79. The average apparent digestibility for lucerne protein as reported in Table 3 is 75 and the true digestibility 91. Our figure approaches that of Satola but is much higher than 56 as reported by Turk and his co-workers.

The addition of cystine to lucerne has no effect on the utilization of the lucerne nitrogen. The average biological value under these conditions is 58, a value very similar to that obtained when lucerne was fed alone. In fact the addition of cystine depresses the apparent digestibility of lucerne protein. This effect also shows up in the true digestibilities. In comparison with the first period of lucerne feeding there is a marked increase in the nitrogen elimination in the faeces when cystine was added. Whether this is due to an incomplete absorption of cystine or an inhibitory effect of cystine on the other nitrogenous constituents, which normally would have been absorbed, is difficult to say. It is, however, evident from these results, that sheep when fed on an 8 per cent. lucerne protein do not need extra cystine. These sheep as was stated were mature wethers and would consequently not have put on new tissue. It may therefore be quite possible that a more liberal need for cystine may occur during periods of active growth.

Furthermore, although this work shows that mature sheep fed on lucerne do not need cystine for maintenance and wool production, it does not exclude the possibility of synthesis of this animo acid. Nevertheless from a practical point of view these results are of great value since they establish that the utilization of lucerne protein for maintenance and wool production is not impaired by a deficiency of cystine.

CONCLUSIONS.

1. By means of the balance-sheet method, it has been shown that the biological value of lucerne protein for sheep is 60 and that the addition of cystine does not effect this biological value.

TABLE 3.

Metabolism Data and Calculation of Biological Value (Daily Basis).

Animal No.	Initial Weight.	Final Weight.	Average Weight.	Dry Matter Consumed.	N Intake Daily.	N in feces Daily.	Metabolic Fecal N.		Food N in feces.	N Absorbed.	Total Uninary N.	Endogenous N.		Food in Urine.	N Retained.	Biological Value.
							Per Gm. Dry Matter.	Per Day.				Per Kg. Weight.	Per Dry.			
	Kg.	Kg.	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
N FREE RATION.																
1............	36	31	33	253	—	·756	·0030	—	—	—	1·20	·036	—	—	—	—
2............	35	35	35	324	—	1·00	·0031	—	—	—	1·70	·048	—	—	—	—
3............	37	35	36	308	—	·677	·0022	—	—	—	1·26	·035	—	—	—	—
5............	40	44	42	461	—	1·29	·0028	—	—	—	1·39	·033	—	—	—	—
8 PER CENT. LUCERNE RATION.																
1............	31	32	31·5	523	8·29	2·44	·0030	1·57	0·87	7·42	3·91	·036	1·13	2·78	4·64	63
2............	34	35	34·5	511	7·82	1·79	·0031	1·58	0·21	7·61	4·21	·048	1·66	2·55	5·06	66
3............	35	35	35	523	8·41	2·20	·0022	1·15	1·05	7·36	4·62	·035	1·23	3·39	3·97	54
5............	41	43	42	461	7·33	1·53	·0028	1·29	0·24	7·09	4·58	·033	1·39	3·19	3·90	55
															Average............	60
N FREE RATION.																
1............	34	32	33	320	—	·960	·0030	—	—	—	1·10	·033	—	—	—	—
2............	35	35	35	270	—	·837	·0031	—	—	—	1·40	·040	—	—	—	—
3............	35	37	36	400	—	1·16	·0029	—	—	—	1·29	·033	—	—	—	—
5............	44	40	42	350	—	·980	·0028	—	—	—	1·39	·033	—	—	—	—
8 PER CENT. LUCERNE RATION PLUS ·20 PER CENT. CYSTINE.																
1............	34	36	35	516	8·13	3·47	·0030	1·55	1·92	6·21	3·56	·033	1·16	2·40	3·81	61
2............	36	36	36	516	8·13	3·33	·0031	1·60	1·73	6·40	3·80	·040	1·44	2·36	4·04	63
3............	35	37	36	516	8·13	2·94	·0029	1·50	1·44	6·69	4·28	·033	1·19	3·09	3·60	54
5............	42	42	42	516	8·13	2·88	·0028	1·44	1·44	6·69	4·57	·033	1·39	3·18	3·51	52
															Average......	58

2. The apparent digestibility of lucerne protein has been found to be 75 per cent. and the true digestibility 91 per cent. The addition of cystine depresses the apparent digestibility and to a slight extent the true digestibility, the average figures being 61 and 80 per cent. respectively.

3. It has been shown that cystine is not a limiting factor in lucerne protein when fed to mature sheep.

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Plant Proteins. VI.—The Amino Acid Deficiencies in Certain Plant Proteins.

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ALTHOUGH the majority of the amino acids were discovered prior to 1900, very limited data exist concerning their relative distribution. Consequently there has been little appreciation of the fact that the nutritive value of a protein depends upon the kind and quantities of its component amino acids. It is, therefore, not surprising that the protein requirements are most generally expressed in terms of amounts of digestible protein without any reference to the differences in quality. However, the biological value of proteins, as developed by Mitchell (1929), takes into account the qualitative differences existing amongst proteins, since it measures the magnitude of absorbed nitrogen actually utilized by the body. It is, therefore, a necessary and essential measure in the calculation of the nett efficiency of different proteins for maintenance, growth, etc. Two proteins may contain the same amount of digestible protein, the only difference being, that one has a biological value of 40 while the other has a biological value of 80. Disregarding qualitative differences one would feed exactly the same amount of the two digestible proteins to cover a specific need of an animal. However, when the quality of these two proteins is taken into consideration, it is obvious that only half the amount of digestible protein of the latter protein is necessary to fulfil the same function as the full amount of the former.

While the biological value actually takes into account the deficiencies of indispensable amino acids in the protein molecule, it does not supply direct information as to the nature of such deficiencies. In order, therefore, to accomplish supplementation judiciously, thereby enhancing the quality of deficient proteins, it becomes imperative that the nature of the amino acid deficiencies prevailing in our feeds should be determined.

Unfortunately no direct and suitable method of determining the amino acid content of feeds is available. Chemical analysis is not only handicapped by laborious, and in many ways incomplete, methods of determination, but also by the fact that such figures, while indicative, have no actual biological significance unless they are tested out on animals. As a result the method most generally employed is very indirect and of the "*hit and miss*" type, by which different amino acids suspected to be deficient are incorporated with a protein and the effect studied by differences in growth response under controlled conditions.

Rose (1935, 1937), through his extensive studies, has in recent years considerably augmented the existing list of indispensable amino acids. Apart from threonine (α amino β hydroxy butyric acid) no new indispensible amino acid has been added. However, valine, phenylalanine, leucine and isoleucine, which have in the past been considered dispensable are now classified by Rose (1937) as indispensable, making the problems of determining the amino acid deficiencies in feeds even more complicated.

As far back as 1915 Osborne and Mendel (1915) demonstrated that *l*-cystine supplements stimulated the growth of rats on a casein containing diet of low sulphur. Since then many investigators have contributed confirmatory evidence of the indispensability of cystine. Johns and Finks (1920) found that the addition of cystine to diets containing phaseolin markedly improves the nutritive quality of this protein. Similarly Sherman and Merril (1925) found that cystine enhanced the growth promoting power of whole milk powder overdiluted with starch. Mitchell and Smuts (1932), in their studies on meat protein, showed that while 20 per cent. meat protein was adequate in cystine, a significant stimulation of cystine on growth occurred when this protein constitutes 9 per cent. of the ration. The same workers (1932), as well as Shrewsbury and Bratzler (1933), and Hayward, Steenbock and Bohstedt (1936), found that cystine was the limiting amino acid in soyabeans. Indeed, so marked is the growth response of rats to the inclusion of cystine in an otherwise adequate ration that Sherman and Woods (1925) actually employed it as a method for quantitative determination of this amino acid. Recently, however, Rose (1937), Beach and White (1937), and Baernstein have produced evidence showing that methionine instead of cystine is the indispensable amino acid. Such evidence must await further confirmation, since it may be possible that these two amino acids may have a reciprocal function in nutrition.

By using Zein as the sole protein in a ration for rats, Osborne and Mendel (1914) demonstrated in a decisive manner the indispensability of tryptophane and lysine. The addition of tryptophane to such a ration satisfied the maintenance requirements, but growth was actually only attained when lysine was incorporated.

Several investigators found soyabeans deficient in cystine. However, the supplementary effect of this amino acid on soyabean protein was repeated, since the possibility existed that all species were not necessarily deficient in cystine. Morris and Wright (1933) found peanutmeal deficient in lysine for milk production, while Johns and Jones (1917), by means of chemical analysis, found no such deficiency. Consequently lysine appeared to be a possible limiting factor in the protein of peanutmeal. No indication of any definite amino acid deficiency in linseedmeal and coprameal was available, so that cystine, the most easily available amino acid, appeared to be the most reasonable to try. The outcome of these different tests is reported below.

EXPERIMENTAL.

The protein feeds which were of an average commercial quality, were finely ground and mixed with the rest of the ingredients of the ration so as to give approximately 8-10 per cent. of protein. The amino acids were incorporated at the rate of ·20 per cent., replacing an equivalent amount of protein nitrogen. These amino acids were carefully mixed with the rest of the ration so as to ensure homogeneous distribution. The percentage composition of the rations is given in Table 1. The paired feeding method was used throughout these studies. In some cases more than six pairs of rats were used, but never less. The two rats of each pair received identical amounts of food, the supplemented ration in one case and the unsupplemented ration in the other. Continuous records were kept of all refusals of feed, which necessitated a reduction in the amount weighed out to both members of the pair. Rats were paired according to age, sex, litter and weight. Frequently rats weighing more than the required weight (60 gms.) had to be used due to scarcity of rats. Body weights were taken weekly and the initial and final weights were the average of three consecutive daily weighings.

RESULTS.

The results of the paired feeding experiments are summarized in Tables 2 and 3. Any significance attributed to treatments must naturally be based on the differences in total gains of pair mates over the experimental period. The size of these differences and the consistency with which they appear determine the probability of the conclusions that they favour. The statistical analyses were performed in accordance with the method of Student (1908) for the interpretation of paired experimental data. These calculations are summarized in Table 4. The probability (P) is obtained from the ratio of the mean difference between pair mates to the standard deviation of differences and by the number of observations. The value of P naturally indicates the significance of the outcome of a treatment. Hence if P is equal to ·01, it may for all practical purposes be concluded that the result obtained is due to the treatment applied and not to a fortuitous outcome due to chance. The greater P becomes the more likely it is that the outcome is due to chance alone.

The supplementing effect of tryptophane on peanutmeal has been tested out on 9 pairs of rats. As will be seen from these results tryptophane supplementation had no effect on the growth promoting properties of peanutmeal, since out of the 9 pairs in the comparison 7 favoured the unsupplemented ration, while only 2 favoured the supplemented ration in total gains in weight. In fact, the probability P strongly favours the unsupplemented ration, indicating that the addition of tryptophane had in all probability exerted a depressing effect on the ration.

In the case of peanut meal supplemented by lysine negative results were also obtained. Out of the 11 pairs in the comparison, 6 favoured the unsupplemented and 5 the supplemented ration in total gains in weight. The probability P of ·28 for the comparisons of total gains is so large that chance alone might well have determined the outcome.

Table 1.

Percentage Composition of the Rations.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Peanutmeal	15·7	15·3	15·7	15·3	15·5	15·2	—	—	—	—	—	—
Coprameal	—	—	—	—	—	—	33·0	32·0	—	—	—	—
Linseedmeal	—	—	—	—	—	—	—	—	26·6	26·0	—	—
Soyabeanmeal	—	—	—	—	—	—	—	—	—	—	35·2	34·7
Cystine	—	·20	—	—	—	—	—	·20	—	·20	—	·20
Tryptophane	—	—	—	·20	—	—	—	—	—	—	—	—
Lysine	—	—	—	—	—	·20	—	—	—	—	—	—
Sucrose	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0
Butterfat	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0	8·0
Codliver oil	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Harris yeast	—	—	—	—	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Yeast Extract (1)	10·0	10·0	10·0	10·0	—	—	—	—	—	—	—	—
NaCl	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
Agar	—	—	—	—	2·0	2·0	—	—	—	—	—	—
Salt mixture (2)	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5	4·5
Starch	48·8	49·0	48·8	49·0	55·0	55·1	39·5	40·3	45·9	46·3	37·3	37·6
TOTALS	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0	100·0
Percent. N	1·50	1·49	1·42	1·41	1·45	1·45	1·40	1·40	1·96	1·93	2·04	1·96

(1) Yeast extract was prepared according to the method of Itter S, Orent E. R., and McCollum, E. V. (*J. Biol. Chem.*, Vol. 108, No. 2, pp. 571-577, 1935).

(2) A modified Osborne and Mendel salt mixture described by P. B. Hawk and B. L. Osler, 1931. *Science*, Vol. 74, p. 369.

TABLE 2.

The Supplementing Effect of Tryptophane, Lysine and Cystine on the Proteins of Peanut.

	8 PER CENT. PEANUT PROTEIN.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.
Initial weight gm.	102	105	96	96	73	76	72	72	105	102	84	84
Final weight gm.	134	130	120	115	105	102	104	100	147	142	129	130
Total Gains gm.	32	25	24	19	32	26	32	28	42	40	45	46
Total food consumption gm.	314	314	286	286	258	258	246	246	343	343	343	313

	Pair 7.		Pair 8.		Pair 9.	
	Control.	Tryptophane.	Control.	Tryptophane.	Control.	Tryptophane.
Initial weight gm.	93	95	80	92	75	67
Final weight gm.	129	128	108	116	102	100
Total Gains gm.	36	33	28	24	27	33
Total food consumption gm.	336	336	322	322	323	323

TABLE 2—(*continued*).

	8 PER CENT. PEANUT PROTEIN.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.
Initial weight gm................	79	79	64	63	52	52	49	49	75	76	55	57
Final weight gm................	130	126	122	115	112	119	95	94	132	122	110	118
Total Gains gm................	51	47	58	52	60	67	46	45	57	46	55	61
Total food consumption gm.......	427	427	427	427	415	415	337	337	427	427	406	406

	Pair 7.		Pair 8.		Pair 9.		Pair 10.		Pair 11.	
	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.	Control.	Lysine.
Initial weight gm................	50	52	50	50	46	48	48	47	63	60
Final weight gm................	66	65	62	67	56	60	67	59	90	88
Total Gains gm................	16	13	12	17	10	12	19	12	27	28
Total food consumption gm.......	176	176	151	151	156	156	167	167	172	172

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm................	81	71	76	76	65	62	135	138	67	69	64	64
Final weight gm................	106	91	98	104	90	88	170	175	89	93	96	104
Total Gains gm................	25	20	22	28	25	26	35	37	22	24	32	40
Total food consumption gm.......	360	360	356	356	342	342	497	497	372	372	349	349

TABLE 3.

The Supplementing Effect of Cystine on Rations containing Coprameal, Linseedmeal and Soyabeanmeal.

	8 PER CENT. COPRAMEAL.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm................	115	107	75	75	85	86	79	79	70	68	73	74
Final weight gm................	162	172	137	135	154	150	117	130	134	141	114	114
Total Gains gm.................	47	65	62	60	69	64	38	51	64	73	41	40
Total food consumption gm.......	512	512	442	442	492	492	405	405	481	481	407	407

	8 PER CENT. LINSEEDMEAL.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm.	99	97	102	104	90	90	95	95	87	88	97	100
Final weight gm................	190	200	188	199	190	187	187	198	180	195	187	192
Total Gains gm.................	91	103	86	95	100	97	92	103	93	107	90	92
Total food consumption gm.......	555	555	555	555	531	531	555	555	555	555	555	555

	8 PER CENT. SOYABEANS.											
	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.	Control.	Cystine.
Initial weight gm................	108	110	94	92	100	99	84	82	80	80	105	106
Final weight gm................	158	187	150	177	160	175	120	149	131	142	154	178
Total Gains gm.................	50	77	56	85	60	76	36	67	51	62	49	72
Total food consumption gm.......	361	361	361	361	361	361	352	352	330	330	361	361

TABLE 4.

Statistical Results of Paired Feeding Experiments.

	CYSTINE.				LYSINE.	TRYPTOPHANE.
	Peanut-meal 8 per cent.	Copra-meal 8 per cent.	Linseed-meal 8 per cent.	Soyabean-meal 8 per cent.	Peanut-meal 8 per cent.	Peanut-meal 8 per cent.
Total gain in weight gm.—						
Mean differences M	+2·3	+5·3	+7·5	+22·8	−1·0	−2·7
Standard deviation S	4·5	9·3	6·7	7·9	5·8	4·0
Ratio M/S (Z)	·51	·57	1·1	2·9	·17	0·67
Probability (P)	·157	·118	·028	·0007	·281	·041
Comparisons of weekly gains in weight—						
No. favouring supplemented ration	15	25	26	38	27	14
No. favouring unsupplemented ration	16	16	13	5	33	30
No. favouring either ration	5	1	9	5	6	10

This finding is not in agreement with that of Morris and Wright (1933), who found peanutmeal seriously lacking in lysine for milk production. However, it is quite possible that milk production calls for an increase of quantity of this amino acid so that peanutmeal may under these conditions be short of lysine. On the other hand, Johns and Jones (1917), by means of chemical analysis, found no deficiency of lysine in the proteins of peanutmeal.

When peanutmeal is supplemented by cystine the result is negative. Although 5 of the 6 pairs show a greater total gain in weight when cystine is incorporated, these gains are not large enough to make the difference statistically significant. The probability P is equal to ·157, a value which is far greater than the accepted standard of significance. Under these conditions it must be concluded that chance alone could have produced the outcome. If, however, pair 1 is omitted then cystine supplements peanutmeal significantly. After our results had been obtained Beach and White (1937) and Baernstein (1938) showed that metheonine was the limiting amino acid in arachin, one of the proteins of peanut. Conarachin the other protein, is apparently complete. Hence it would appear that methionine is the limiting indispensable amino acid in peanutmeal.

In Table 3 the supplementary effect of cystine on coprameal, linseedmeal and soyabeanmeal has been summarized. Of the 6 pairs of rats on the coprameal and coprameal supplemented by cystine, 3 favoured the supplemented and 3 the unsupplemented ration as regards total gain in weight. In the 42 weekly comparisons of gain in weight, rats, on the supplemented ration, gained 25 times and those on the unsupplemented ration only 16 times. However, the probability P, that the greater gain of the supplemented ration has been a chance outcome is ·118, a value several times greater than the critical value of ·03. It is, therefore, concluded that the difference in total gain cannot be attributed to the effect of cystine.

In a comparison of linseedmeal with linseedmeal supplemented by cystine, 5 out of the 6 pairs favoured the supplemented ration in total gain. The probability (P) that this difference in total gains is due to chance is only ·028, which means that the chances are approximately 34 to 1 that chance alone would have brought about such a result. It is clear, therefore, that cystine has exerted a supplementing effect on the proteins of linseedmeal.

That cystine has a definite, supplementary effect on the proteins of soyabeans is seen from Table 3. Of the 6 pairs in the test all responded to treatment of cystine and outgained by far their pair mates on the unsupplemented ration. The probability P that this result is due to chance is equal to ·0007, which clearly demonstrates that this outcome is exclusively due to the inclusion of cystine in the ration.

SUMMARY AND CONCLUSIONS.

By means of the paired feeding method, the possible indispensable amino acid deficiencies of peanutmeal, coprameal, linseedmeal and soyabeanmeal have been investigated.

It has been found that peanutmeal is not deficient in cystine, tryptophane or lysine, but may be in the light of investigations which appeared after the work had been completed, deficient in methionine. Coprameal is not deficient in cystine, while linseedmeal and soyabeanmeal are definitely deficient in cystine.

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Section VI.

Haematology.

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The Blood of the Ostrich.*

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INTRODUCTION.

BLOOD is subject to many diseases and the study of haematology not only yields information concerning these diseases but often also furnishes valuable information for use in the interpretation of disorders which primarily affect other parts of the body. However, all knowledge about the state of the blood during disease is useless without comparative data for healthy blood.

The purpose of this treatise is to present haematological data for use in the study of diseases of the ostrich, and to render possible a comparison of the data on ostrich blood with those on the blood of other animals.

In order to avoid repetition, the technique, results and discussions of each phase of the investigation are grouped together, whenever possible, and the work is presented under the following headings:—

1. Subjects used for determinations.
2. Marking of ostriches.
3. Collecting the blood.
4. Preventing the coagulation of the blood.
5. Preparation of smears.
6. Staining of smears.
7. Cell measurements.
8. Morphology of the blood.
9. Erythrocyte counts.
10. Relative volume of corpuscles and of plasma.

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11. Minimum and maximum resistance of red cells.
12. Osmotic pressure on the red cells.
13. Haemoglobin content.
14. Leucocyte counts.
15. Differential counts.
16. Thrombocyte counts.
17. Viscosity.
18. Specific gravity.
19. Phosphorus, calcium, sodium, magnesium and potassium content of the blood.
20. Total blood volume.
21. General discussion.
22. Summary.

The blood of birds has been little investigated compared with that of domesticated mammals, and literature referring to the blood of the ostrich is very meagre indeed. Table 1 comprises all the records (relating to the blood of this bird) revealed by an exhaustive search carried out with the assistance of the Staff of the Imperial Bureau of Animal Health, Weybridge, Surrey, England. None of the six investigators mentioned in Table 1 indicated the technique employed for collecting the blood, neither was there any mention of the number of birds from which blood was collected, nor the number of blood samples examined.

It would appear, therefore, that the data were obtained incidentally in the course of other studies; and it will be observed that, although the erythrocyte counts given by Malassez (1872) and Hayem (1879 and 1889) are in fairly close agreement, they differ considerably from that of Venzlaff (1911). The cell lengths recorded by Malassez and Venzlaff also differ appreciably from those given by Gulliver (1875) and Hayem. But in any case, great accuracy can scarcely be expected in the cell counts and cell measurements by the early investigators, considering that in those days the apparatus was not so accurately calibrated as it is to-day.

The ostrich is a domesticated bird with a very ancient history and the following information given by Wormser (1930) may prove of interest. A specimen of the bird was found in a sepulchral chamber of the 18th dynasty which is supposed to be contemporary with Moses and frequent mention of the feather is made in Egyptian hieroglyphics. Arsenoe, an Egyptian queen before Cleopatra, caused to be erected a statue of herself seated on an ostrich, and in Roman public functions the feather was much worn and the bird was ridden by ladies of noble birth. The beauty of the feather has been praised in all ages and from time immemorial the plumes have been sought

Author and Date.	ge ar nents. Breadth.	Phosphorus Content.		Agglutination titer of anti-lepisepticus Serum.	Agglutination titer of anti-pigeon R.B.C. Serum.
iez, L., 1872......	—	—		—	—
ır, G., 1875........	$\frac{1''}{9166}$ 2·77μ	—		—	—
, G., 1879.........	—	—		—	—
, G., 1889.........	—	—		—	—
f, W., 1911........	—	—		—	—
A. I., 1930........	—	—		—	—
		Total Phosphorus......	109·0	—	—
		Lipoid Phosphorus.....	11·3	—	—
		Organic Phosphorus....	39·0	—	—
		Inorganic Phosphorus..	5·5	—	—
		Nucleo-protein.........	53·2	—	—
ıder, 1934.........	—	—		0	40

the clotting of blood.

Author an		Percentages of Varieties.				
	ytes.	Monocytes.	Polymorphonuclears with Eosinophilic Rods.	Polymorphonuclears with Eosinophilic Granules.	Basophiles	Unclassified.
Stolzing, 1856(1).......		—	—	—	—	—
Malassez, 1872(1)......		—	—	—	—	—
Hayem, 1889(1).......		—	—	—	—	—
Albertoni and Mazzoni,		—	—	—	—	—
Moore, 1895–6(4), (7)...		—	—	—	—	—
Heinz, 1901(7).........		—	—	—	—	—
Ward, 1904(4).........		—	—	—	—	—
Warthin, 1907.........		—	21·5	10	2	16·5
	·5)					
	·5)					
Ellerman and Bang, 19		23	37		—	—
Hirsfeld, 1909(4), (7)...		—	22·5	—	—	—
Goodall, 1910(1), (7)....		—	37	6	1	—
Hedfeld, 1911(4), (7)...		—	30·0	12·0	3·0	—
		—	—	—	—	—
Kleineberger and Carl,		—	29·5	4·5	2·2	—
	·3)					
	·5)					
Burckhardt, 1912......		—	27·9	7·9	2·9	—
	·5)					
	·8)					
Kozma, 1913..........		—	—	—	—	—
Launoy and Levy-Brul		—	—	—	—	—
Schmeisser, 1915......		19·4	29·6	4·3	2·2	2·2
Pickens, 1915(5).......		—	—	—	—	—
Fatham, 1916(4).......		—	—	—	—	—
Taylor, 1916(1), (4)....		9	33	3·6	3	—
	)	(6–14)	(25–41)	(1·9–6·8)	(2·6–4·1)	—
Burnett, 1917..........		5·5	28·8	3·3	4·3	—
Mack, (no date) (1)....		6·2	32·7	2·7	3·3	—
Kaupp, 1918(5)........		—	—	—	—	—
Salomon, 1919(3), (4)...		1·0	28	7	3	—
		2·0	18	9	1	—
Fritsch, 1920(3)........		2	49	5	3	—
		5	23	5	2	—
Kleineberger and Carl,		—	60–71·5	1–2	0·5–14·0	—
		—	18·6–33·5	1·5–4·0	1·5–3·5	—
Ellerman, 1921........		12	29		4	—
Romer, 1921..........		1·4	26·5	5·4	1·9	—
	·7)					
	·3)					
ink, 1924(2), (7)......		—	—	—	—	—

[*Continued on next page.*

Cells per c.mm. (Thousands).		Thrombocytes per c.mm. (Thousands).	Percentages of Varieties.					
Minimum.	Average.		Lymphocytes.	Monocytes.	Polymorphonuclears with Eosinophilic Rods.	Polymorphonuclears with Eosinophilic Granules.	Basophiles.	Unclassified.
19	29	—	52·5 (40·4–64·7)	7·5 (4·3–10·6)	32·1 (24–41)	6 (3·7–10)	1·9 (0–3·4)	— —
—	33·0	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
22	38·9	—	81	1·5	5·17	10	1·6	—
—	33·3	—	66·5	9·2	17·7	4·1	2·5	—
10·1	18·6	None	32·8	5·7	49·4	8·7	3·6	—
14·0	17	50–100	—	—	—	—	—	—
—	—	—	58·1 (41·6–69·9)	9·1 (4–15·6)	29·1 (18·8–41·0)	1·4 (0–6·0)	2·3 (0–5·0)	— —
8·0	—	—	—	—	—	—	—	—
6·8	24·6	35·0	41·8	17·1	34·7	1·8	4·2	—
25·0	—	50·0	45·0	18·0	30·0	6·0	1·0	—
—	20·0	—	—	—	—	—	—	—
—	30·0	—	65·0	5–10	25·0	2–4	1–2	—
—	—	—	60·0 (small 40–60) (large 5–15)	2 (1–4)	30 (20–50)	5 (2–8)	3 (1–5)	—
—	—							
19·8	24·4	—	—	—	—	—	—	—
—	27·0	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
3·0	16·36	—	12–58 (small 8–54) (intermediate 0–18) (large 0–5)	4–33	22–78	0–18	0–18	0–3
—	—							
—	24·0	—	—	—	—	—	—	—
13·3	25·1	—	69·4	7·5	21·5		—	1·6
15·8	38·5	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
18·33	32·15	—	—	—	—	—	—	—
15·00	20·60	—	—	—	—	—	—	—
—	19·8	25·7	62·5	9·4	24·4	1·9	1·7	—
—	32·7	29·4	66·0	8·1	20·9	1·9	3·1	—

osinophilic leucocytes with rods 10·3 per cent. Eosinophilic leucocytes with granules 23·0 per cent.
ilic leucocytes and mast cells 4·2 per cent. Small lymphocytes 40·3 per cent. Large lymphocytes

(9) Adult fowls.

(10) Young chickens.

for personal adornment. Warriors wore them as early as 1350-1100 B.C. and ostrich eggs were greatly prized and used for ornamental and religious purposes. The plumes formed part of the tribute imposed by Egyptians on conquered countries.

In the light of these facts and in view of the important role which the ostrich has already played in the economic history of South Africa it is surprising that the haematology of this bird has not received more attention, particularly, from veterinarians in South Africa and that there is comparatively little scientific veterinary literature about the ostrich. This lack of information cannot be ascribed to the bird's immunity from disease, for, though the grown animal is very hardy, yet when young—particularly when under three months—it readily succumbs to disease.

That it is susceptible to a number of affections is well known, though to judge by the literature only a few of these have been scientifically investigated. This may be attributed partly to the fact that countries other than South Africa had little interest in the ostrich feather industry; for, during the years in which it flourished, it was almost entirely monopolised by South Africa, since the bird is indigenous to this country, and legislation prohibited the exportation of ostriches or even of fertilised eggs. Moreover at that time there were few veterinarians in South Africa and they were fully occupied with the many stock diseases then rampant, which owing to the havoc they were causing doubtless warranted more attention. Apparently no one qualified to do so had sufficient leisure to make a thorough study of the hygiene and diseases of the ostrich and to present the information for the guidance of others. Besides, veterinary haematology and especially avian haematology was then much less advanced than it is at present.

For almost half a century the ostrich industry was of very great importance to South Africa and formed the most lucrative branch of farming. At one time ostrich feathers were second only to wool as an agricultural export. From the year 1860, when 2,361 lbs. of feathers valued at £19,726 were exported from South Africa (Wormser 1930), the exports steadily increased, except for minor fluctuations, till 1913, the peak year of the industry, when 1,023,307 lbs. valued at £2,953,587 were exported. At the end of 1913 there were 776,313 ostriches in South Africa, and before the collapse of the industry in 1914 as much as £1,000 was paid for a good breeding pair, and the prices obtained for chicks varied from £5 to £50 (Official Year Book of the Union of South Africa, 1910-'17). Feathers realised as much as £100 per lb. during the boom (Laite 1915).

The ostrich feather industry forms at present one of the lesser branches of South Africa's agricultural activities, and may be said to have been moribund for many years in so far as the value of the feather as an article of adornment is concerned. Still, it does not seem likely that ostrich feathers will ever pass quite out of favour, though the price may fluctuate in accordance with the vagaries of fashion. The ostrich is being exploited also in other ways—its skin is used for wallets, handbags, attache cases, tobacco pouches, shoes, etc., and the meat, particularly when dried (biltong), and the eggs are relished by many people. The following paragraph recently appeared in the press:—

> "Having discovered that ostrich meat has a delicious flavour, the Moscow Zoological Park has begun breeding ostriches on a large scale, with the object of establishing large flocks in the Southern Steppes of the Soviet Union. Because of its thick layer of fat, which originally served as a protection from the Semi-tropical sun, the ostrich can endure the cold Russian winter, even at the latitude of Moscow."

The knowledge gained from veterinary research work in connection with the ostrich would, therefore, serve the interests not only of science but of trade, especially if there should be a revival of the feather industry. It is hoped that these studies will add a link to the chain of that knowledge.

Incidently it may be mentioned that the literature discloses no data about the blood of any kind of South African bird—apart from the chemical analyses by Malan (1930)—and it will be seen from Table 2, which embraces most of the observations on the cellular elements of the normal blood of the domestic fowl, that the results show marked discrepancies, especially in respect of the total leucocyte and thrombocyte counts and the percentages of the types of leucocytes. Moreover, blood observations made overseas are not necessarily applicable to South African birds, for, as is well known, the blood picture varies more or less with breed, climate, geographical habitat, etc.

SUBJECTS USED FOR DETERMINATIONS.

As the primary object of this work was to obtain information about the blood of normal ostriches the intention was to utilise, for the purpose of research, birds hatched either in an incubator or naturally and removed immediately to an area where there was no risk of worm infection—to which the ostrich is very susceptible—and where they could be so attended to as to remain in perfect health. But incubator-hatched birds were unobtainable and the writer had to content himself with six chicks which had been hatched by a semi-wild ostrich, and which were already about two weeks old when they were caught. Luckily they proved later to be three hens and three cocks and consequently the blood of the sexes could be compared.

Before capture these birds had the run of a big farm on which there were few other ostriches and it was, therefore, considered that little, if any, worm infection could have occurred within so short a period. They were then reared in a camp on the Stellenbosch-Elsenburg Agricultural College farm, Mariendahl, Stellenbosch district, where they had considerable freedom and where there had been no ostriches for many years (perhaps never), not even within a radius of many miles. Furthermore, as the four species of worms known to be harboured by the ostrich, viz., the tape-worm, *Houttuynia struthionis*, and the nematodes, *Codiostomum struthionis*, *Ornithostrongylus douglasii* and *Contortospiculum spicularia* (Mönnig 1929 and 1934), are not known to occur in any other species of animal, little, if any, worm infestation was anticipated. This expectation was fortunately realised, for faeces examinations made—by the centrifugal flotation method described by Mönnig (1934)—during the course of the research work proved negative and also on post-mortem examination no worms were found. The administration of vermicides was, therefore, unnecessary, and possible blood changes due to such treatment was accordingly eliminated. Care was taken also to ensure that the birds remained entirely free from ectoparasites. Five of the birds—numbers 1 to 5—showed no trace of disease, either during life or on post-mortem examination, and their blood may, therefore, be considered to have been normal.

The remaining bird, number 6, when about six months old, dislocated a metacarpo-phalangeal joint and the dislocation could not be remedied. In consequence the bird had a club-foot, and as the joint always came in contact with the ground a large tumour-like formation developed over the part exposed to the ground. Otherwise the bird was perfectly healthy, for on post-mortem examination it showed no internal evidence of disease. As it could not be considered perfectly normal, the results of the examinations of its blood are not included with those from the normal birds, but they are nevertheless recorded for the purpose of comparison.

It would, of course, have been better to have had a larger number of absolutely healthy birds for these blood observations, but sufficient lucerne-grazing, so essential to the well-being of ostriches kept in a limited area, was not available. Their other food consisted of a plentiful supply of chopped bones, oats, mangolds, etc. Some of the observations, such as cell-counts, etc., were repeated at intervals from the time when the birds were about seven months until they were about two years and eight months, viz., until they were fully mature.

The blood of sixteen other ostriches was also examined. Eleven of these—numbers 7 to 17—were semi-wild full-grown birds caught on the farm " Nagwag ", Bredasdorp District. They appeared healthy, but the writer was unfortunately not given an opportunity of conducting post-mortem examinations on them. Faeces from only a few of these birds could be examined and these contained many worm eggs. As none of the birds had ever received vermicidal treatment, it is most likely that all of them were worm-infested.

The remaining five birds, 18 to 22, were semi-wild ostrich chicks sent to the writer from the farm " van der Stelkraal ", Bredasdorp District. These birds showed marked unthriftiness, and on post-mortem examination they were all found to be severely infested with worm parasites, particularly tapeworms. As it is impossible to judge of normality from external appearance alone, only the results from birds 1 to 5 will be considered as results from normal birds, and those from numbers 7 to 17 and from numbers 18 to 22 will be classed respectively as from clinically healthy ostriches and from unthrifty ostrich chicks which on post-mortem examination showed marked verminosis.

It was difficult to make observations as the nearest farm—other than Mariendahl—on which ostriches were kept was over a hundred miles distant from the laboratory.

CLASSIFICATION OF THE OSTRICH.

There are different opinions regarding the classification of the ostrich. Cronwright-Schreiner (1898) gives the following classification:—

1. North African bird—*Struthio camelus.*
2. South African bird—*Struthio australis.*
3. Somali Ostrich—*Struthio molybdophanus.*

In South Africa the industry was started with the *Struthio australis*, but as the feather of the North African bird, *Struthio camelus*, proved superior, a number of these birds were introduced at different times to improve the stock. Cross-breeding was successful and, as the hybrids are fertile, a certain percentage of the birds in South Africa are crosses between the two species, *Struthio camelus* and *Struthio australis.* The birds used in this work, however, had the characteristics of the true South African bird, namely, *Struthio australis.*

THE MARKING OF OSTRICHES.

As ostriches look so much alike—even the sexes being difficult to distinguish until the birds are about a year old—it was necessary to mark the birds on which repeated observations were being made, so that they could be readily identified. They were, therefore, branded, for branding is a simple and most effective way of marking an ostrich. Clips in the wing were considered undesirable, as they sometimes cause inflammation and possibly leucocytosis.

A piece of wire, about 7 mm. in diameter and 18 inches long, was bent at right angles, so that the short arm of the wire was two and a half inches long. The six birds kept at Mariendahl were each branded on the right thigh in Roman letters, viz., I to VI, when they were four months old and the letters were placed sufficiently low and were big enough to be seen readily from a distance; thus any unnecessary handling of a bird was avoided. The wire, heated to a dull red, was applied just long enough to burn through the epidermis. Excessive burning, particularly when a small brand with ordinary lettering is used, sometimes results in so much scar tissue formation that the actual lettering is obliterated. If chicks under three months have to be branded, it is best to brand them on the side of the abdomen, for the thighs are then still covered with down. A fine wire should then be used. Healing is usually complete after five weeks and the mark probably remains for life. Determinations recorded were made only about two months after the wounds had completely healed.

COLLECTING THE BLOOD.

From ostriches up to the age of about four months small amounts of blood, such as for cell counts, are most easily obtained by puncturing the brachial vein just above the shoulder joint. The bird is laid on its side on a table or box of convenient height, and is suitably controlled by one or two assistants. It is much easier to restrain troublesome birds if the hock joints are flexed and then immobilised by means of cords, or preferably soft linen bands, as the skin is easily chafed. The uppermost wing is held extended and the site is cleansed with a pledget of cotton wool moistened with alcohol. The vein, which is easily seen when the wing is extended, is then slightly compressed opposite the shoulder joint with the thumb of the left hand and when the site is quite dry the vessel is punctured, an ordinary hypodermic needle being quite suitable for the purpose. The needle should be held in a position vertical to the course of the vein ; if the puncture is made obliquely, the skin stretches slightly, with the result that when the needle is withdrawn the skin retracts, preventing the free flow of blood. The blood then collects on the fold of the skin which fills the angle between the humerus and the ribs, and the required amount of blood can then be correctly drawn up in a blood cell count or other kind of pipette. Blood cell count pipettes (Thoma-Zeiss and Trenner) were always used for diluting the blood in the case of young chicks, as much blood could not easily be obtained, nor was it desirable to extract much blood. Haemorrhage may be arrested by pressing a finger on the wound for about one minute, but after release of the bird bleeding generally stops quite soon.

When a fair quantity of blood was needed, as for the purpose of determining percentage volume, etc., it was drawn from the brachial vein by inserting into it a hypodermic needle with a bore of about 0·5 mm. and drawing off the required amount of blood with a syringe. As a needle of such fine bore has to be used the blood seldom flows satisfactorily of its own accord even when the vein is well compressed. (Differences between counts made of blood drawn off with a syringe and of blood which flowed out spontaneously were negligible). Very young chicks should be handled carefully as they readily succumb to injury.

In older birds, sufficient blood for ordinary haematological determinations can be obtained from either the right jugular or a brachial vein, for these veins are well developed. The blood was usually collected from the right brachial vein, which is easily accessible; the birds usually resent handling of their necks. They persist in swaying them about and they usually struggle if attempts are made to control the neck. The needle, therefore, easily becomes dislodged. The procedure adopted here was based on the method of Neser (1923) and is as follows:—

The birds are driven into a small enclosure and by means of a stick—about six feet long, to one end of which a thick piece of hard wire bent into the shape of a hook is attached—the bird is gripped at the back of its neck just below the head. The bird immediately rears and with the right hand it is then caught at the back of the neck. Very tame birds may be caught without the aid of a stick. A black stocking or some other type of blinker porous enough to admit sufficient air but too dense for the bird to see through is pulled over the head. When once the head has been caught there is little danger of being kicked, and it is surprising how helpless and docile an ostrich becomes when it cannot see. The bird is then manoeuvred into a V shaped crush just long enough to accommodate it. The back of the ostrich is on a level with the top of the crush. (Fig. 1.)

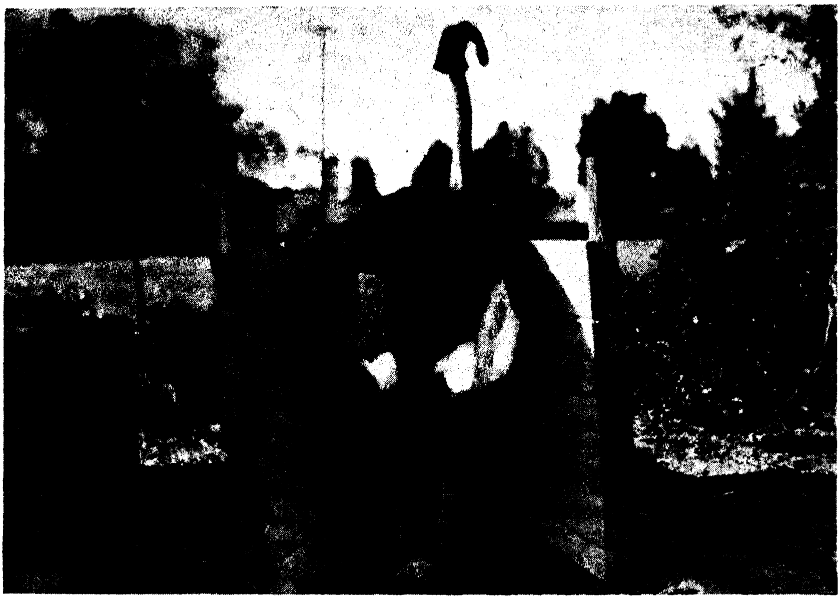

Figure 1.

It is not desirable to use galvanised iron for making the crush, for noise caused by the kicking of the birds frightens them and they become very restless. Neither should the crush be built close to trees for their rustling on a windy day also excites the birds. Flooring boards were found very suitable. Their surfaces should be planed smooth otherwise the ostrich can get a hold on rough surfaces with its claws and thus lever its body backwards and upwards and make control difficult. Thinner boards might be broken by an adult bird.

In the crush the bird is controlled by one or more assistants applying their hands to the ischial bones and pushing the bird forward, while others push him downwards by applying their hands to the sacral region, and at the same time another assistant lifts the right wing. The operator stands on the left side of the bird, and during the operation of inserting the needle into the vein and collecting the blood he presses with his fore-arms on the bird's back thereby also helping to prevent its moving upward. Occasionally a bird will flop down and refuse to get up. It is best then to drag it out of the crush and to press it down so that it cannot get up while the blood is being drawn. At least three men are required to keep an adult ostrich down. Attempts were made to control birds in the crush by tying ropes round them to obviate the use of many assistants, for at least four are required to control a restless bird. This method, however, proved undesirable, for the feathers were easily dislodged and the skin was badly chafed by the ropes.

The semi-wild birds from which blood was collected in the Bredasdorp district, when once they were caught, struggled much less than the six birds kept at Mariendahl, and they did not very vigorously resist the drawing of blood from the jugular vein; no doubt they were too scared to do so. Of the six " Mariendahl " birds five soon became used to being handled and scarcely resented the insertion of the needle, so that less assistance was required. But the remaining bird, No. 6, always put up a struggle.

In an adult ostrich the brachial vein measures about 6 mm. in diameter, and a hypodermic needle about 4 cm. long and with a bore of about 1 mm. in diameter was found quite suitable for insertion into the vein of a full-grown bird. For younger birds a needle with a diameter of 0·7 mm. was used. The wings were used alternately when fairly frequent collections were made and sometimes the blood was obtained from the jugular in order to eliminate the possibility of contamination by inflammatory products. But it would appear that phlebitis is not easily induced in the ostrich. It was never once observed and it is surprising how soon the puncture wound, or indeed any wound, heals in the ostrich.

In the adult bird the skin overlying the vein is fairly thick and its colour is blue like that of the vein; the vessel cannot, therefore, be seen until the blood flow is slightly impeded by gentle compression, which is also necessary in order to obtain a free flow of blood. When the needle is inserted it should be pushed in at a very acute angle and in a direction against the flow of blood, so that its point is at least 0·5 cm. from the point of entry into the vein; otherwise the opposite wall of the vessel may be penetrated or, if not inserted deeply enough, it may slip out if the bird struggles slightly.

A 20 per cent. solution of lithium citrate and powdered heparin, which are again referred to under " Preventing Coagulation of the Blood ", were used as anticoagulants. Lithium citrate was used in the early part of the work, but was later replaced by heparin, and the blood was collected in bottles with necks about 0·5 centimetre in diameter and graduated as described by Neser (1923). Each bottle contained such an amount of the lithium citrate solution, or of the powdered heparin, that when the bottle was filled to the mark on the neck, there was 1 part of lithium citrate to 199 parts of blood, or 1 part heparin to about 5,000 parts of blood. It is not always easy to fill the bottle accurately to the level of the mark but since such a small proportion of anticoagulant is used a slight error in the level is not significant when a fair quantity of blood is drawn. The blood was, however, always collected in such small amounts and at such intervals that the observations may be regarded as always having been made on normal blood.

According to the observations by Neser (1923), the rate of the circulation is a very important factor in the distribution of the red cells, a sluggish peripheral circulation resulting in a concentration of the red cells in the periphery and the reverse in the jugular vein. He considered the jugular vein in domestic mammals the best source from which to collect blood, and his reasons may with advantage be repeated here:—

1. " The whole circuit is comparatively short and the flow of blood is fairly rapid.
2. The circulation of the head is relatively larger than that of any other accessible part of the body, and for this reason the relative change in the blood for a given activity will be least.
3. There is little, if any, obstruction to the flow of blood in the jugular vein owing to the action of gravity. Stagnation is, therefore, impossible here under normal circumstances, and the blood is consequently thoroughly mixed."

Ponder (1934), on the other hand, remarks: " In spite of occasional statements to the contrary, it seems to be established that the red cell count is the same in normal arterial, venous, and freely flowing capillary blood, and that it is the same in normal blood taken from central and peripheral vessels (Rud, 1922-23, McCay, 1928)."

The wing vein of the ostrich may, for the reasons mentioned by Neser, be considered more suitable than the jugular, for the circulation is much shorter in the wing than in the neck, while the other two reasons are equally applicable to the wing vein. Besides, as already stated, blood is more easily obtained from this source.

Counts and percentage volume determinations were made from blood extracted from the jugular and also from the wing vein, but they differed only within limits of error, so that, both for theoretical and practical reasons, the brachial vein may be considered the most suitable in the ostrich.

PREVENTING COAGULATION OF THE BLOOD.

In testing ostrich blood to determine its coagulation time, it was observed that clotting is usually much delayed, and often samples of whole blood, still uncoagulated, were discarded days later. Usually samples did not coagulate for several hours after they were collected but sometimes it did happen that coagulation occurred within an hour. The great advantage of this unusual property of ostrich blood was that observations could also easily be made on whole blood and these could be compared with those made on blood containing anticoagulants.

Bainbridge and Menzies (1919) state: " The blood of birds contains no platelets, and will not clot, if it is drawn directly from a blood vessel without contact with the tissues. If, however, it is allowed to flow over the adjacent ~~tissues~~ in its passage from the vessel, it will coagulate readily."

This statement requires modification, for, though the blood of the ostrich does not readily clot, this is not true of fowl blood. The writer has on many occasions drawn blood—by means of a syringe—direct from the hearts of fowls according to the method of Sloan and Wilgus (1930), and also from their wing veins, and coagulation usually occurred within a short period even when the inside of the syringe barrel was coated with vaseline. According to Dukes (1934) the period of coagulation for fowl blood is 4½ minutes, and Johnson and Connor (1933) who determined the coagulation time of the blood of 12 fowls found that it varied from 1 minute 10 seconds to 14 minutes 15 seconds with an average of 6 minutes 21 seconds. Kaupp (1929) states the period to be 30 seconds. It is, of course, generally agreed that, though platelets as seen in mammalian blood do not occur in avian blood, the thrombocytes in birds' blood serve the same purpose as the platelets.

Ostrich blood sometimes clots quickly, especially in warm weather, once it has been in contact with the tissues or if brought in contact with blood that has already coagulated. It was also noticed that the blood coagulated more readily when the bottles were filled slowly because of obstruction in the needle or when a needle with a very fine bore was used.

It would appear that the prolonged coagulation time of ostrich blood cannot be correlated with a low calcium content of the blood. From Table 29 it will be noted that the calcium content of ostrich blood averages 10·1 mgms. per 100 c.c. blood. This figure is high compared with the values given for some animals whose blood clots very readily. For example—to give only a few figures as recorded by Dukes (1934)—the calcium content of the blood of the ox is 7·1 mgms. per 100 c.c. and the coagulation time 6½ minutes, the corresponding figures for the dog being 6·6 mgms. and 2½ minutes and for the cat 5·5 mgms. and 1 to 3 minutes. Calcium values, reported by Knowles (1934) for hens not in lay, vary from 8 to 12·3 mgms. per 100 c.c. blood.

As coagulation of samples sometimes did take place before it was possible to complete the observations, it was necessary to use an anticoagulant. Neser used a 7½ per cent. sodium citrate solution as anticoagulant in the proportion of 1 part anticoagulant to 9 parts of blood, and the writer's intention was to use, also in the same proportion, an isotonic solution of a suitable anticoagulant so that true percentage volume readings could be obtained. It was, however, soon realised that, whereas it is possible to fill up the bottle containing the anticoagulant to a definite mark when collecting blood from a docile animal like the horse, it is difficult to do so when extracting blood from an ostrich. As a rule, the birds are restless, and as the operator has to collect blood from the under side of the wing while usually standing on the opposite side, it is often impossible to fill up the bottle exactly to the mark. This necessitates the subsequent addition of saline or the removal of blood, and therefore additional calculations have to be made.

It was, therefore, decided to find an anticoagulant of which such a minute quantity need be used, either in solid or liquid form, that corrections need not be made even if the bottle is filled up slightly above or below the mark. Sodium citrate, potassium citrate, lithium citrate, sodium oxalate, potassium oxalate and sodium fluoride were tried in solid form. But it was found that, when these were used in such quantities as would invariably prevent coagulation, slight haemolysis sometimes resulted, as was evidenced by tinged plasma when the blood was centrifuged. The aforementioned salts in powder form were, therefore, considered unsatisfactory, as the degree of haemolysis could not be controlled. It was finally found that 0·5 c.c. of a 20 per cent. solution of lithium citrate to 99·5 c.c. of blood invariably prevented coagulation and never caused any haemolysis. Moreover, as lithium citrate is a salt that does not occur in the blood, corrections need not be made in chemical analyses of the blood.

The lithium citrate solution, however, causes slight crenation of the cells, and consequently a true percentage volume reading is not obtained. But it will be shown under " Relative Volume of Corpuscles and of Plasma " that, by applying a small correction factor to percentage volume readings obtained with citrated blood, such readings may for practical purposes be regarded as identical with those of whole blood.

Heparin, which was unprocurable in South Africa at the time it was first required, was tried later and proved an even more suitable anticoagulant, for, when used in the proportion of 1 mg. to 5 c.c. blood as was used by Wiseman and Bierbaum (1932) for human blood, it also invariably prevented coagulation. Haemolysis was never observed and differences, when present in the percentage volume readings of whole blood and of heparinised blood, were insignificant.

The aforementioned workers state: " After weighing out this quantity a few times to visualise the approximate volume involved, the quantity of heparin added to each tube may be estimated without disturbing the accuracy of the test ". This procedure was adopted by the writer and proved satisfactory.

PREPARATION OF SMEARS.

Opinion differs considerably as to the most satisfactory method of making blood smears suitable for differential counts. According to Neser (1923) the distribution of the leucocytes is often most irregular when smears are prepared by the slide method, viz., by placing a drop of blood on one slide and causing it to spread by pushing it with the edge of another slide. He states that as a rule the leucocytes are evenly distributed throughout the smear by his coverslip method in which the blood is spread between a slide and a coverslip, instead of between two coverslips, as is customary in determinations on human blood. However, he does not record differential counts made from smears prepared in both ways, indicating the differences in the counts. De Kock (1931) slightly modified Neser's method by substituting a squarely cut portion of slide glass for the coverslip and states that the method gave more uniform results than smears prepared in any other way. Many other workers also favour the coverslip method.

On the other hand, Schilling (1935) describes only the slide method. Wirth (1931) is of opinion that the distribution of leucocytes is as even with the slide method as with the coverslip method. Lucia and Lucia (1928) give counts made in different ways from smears prepared by the slide method and state:—

> "It was found that the counts in every case fell within one standard deviation of the group. If the probability of variation around the mean (standard deviation) is taken into consideration, no advantage can be ascribed to the coverslip over the slide method, as far as distribution of cells is concerned."

Kolmer and Boerner (1931) describe both methods, and their only comment is:—"The coverslip method is preferred by many clinical pathologists, although it tends to produce rupture of the leucocytes." In other text books consulted usually both methods were also described, but no preference was mentioned. The writer also consulted several well-known haematologists, and they prepare smears for differential counts only by the slide method.

Messrs. Arthur Thomas & Co., Philadelphia U.S.A. have placed on the market a "Differential Counting and Staining Outfit" invented by Hausser. With this outfit, smears are prepared as in the coverslip method, the blood being spread with a small rectangular block of glass on a specially thick glass slide. The following remarks from the firm's 1931 Catalogue edition, in which the outfit is advertised, may be quoted here: "It is difficult to obtain blood smears of uniform thickness over a large area with any technique when ordinary microscopic slides and coverglasses are used, because of the curvature in the surfaces of both. It has been stated that this usually amounts to a full wave-length in a linear distance of 1mm. Since a wave-length is about 600 microns, and a blood corpuscle measures about 10 microns, it is evident that, while at one point absolute contact may be possible, there may be sufficient unequality of surface at a distance of only 1 mm. from such a point to pile up a layer of not less than sixty corpuscles, when smears are so made."

Therefore, it does not appear to have been definitely established yet that the coverslip method gives a more even distribution of the leucocytes or in any case a so much more regular distribution as to be of practical importance.

Smears may be easily and satisfactorily prepared, by the coverslip method, from the blood of animals which can be brought into a laboratory or sheltered place in which the air can be kept free from dust, but in the case of the ostrich this is not so easy. Usually ostriches are not stabled, but are allowed to pick up their food on the veld, and they can only with difficulty be driven into a stable. Under ordinary field conditions, therefore, smears have to be prepared in the open, and as the surface of the glasses have to be scrupulously clean in the coverslip method it is difficult to prepare such smears on a windy day as the dust so quickly settles on the glasses. Moreover, the numerous fine scales on the feathers of the ostrich easily become dislodged and settle on the glasses.

The coverslip method of Neser as modified by De Kock was tried and, apart from the above-mentioned disadvantage associated with the coverslip method when applied to the ostrich, the writer often had to make a number of smears before a satisfactory one was obtained even on quiet days when the surfaces of the glasses were as clean as could be expected. The slides were always cleaned with bichromate of potash and nitric acid and kept before use in absolute alcohol but owing to the unevenness of the slides—though high quality polished slides were always used—the blood would not spread properly on many of them, and consequently the smears were too thick. Smears could, therefore, not always be prepared quickly with the result that the flow of blood from the needle had to be unduly prolonged—a matter of considerable inconvenience when dealing with a restive animal. Apart from the loss of blood, the bird soon becomes uneasy and the needle is either dislodged or the blood-flow ceases and the needle has to be reinserted.

Thin smears from ostriches can be easily prepared under field conditions by the slide method described by Neser (1923), slightly modified as indicated below;

A platinum loop is attached by means of sealing-wax or other suitable adhesive to the end of the slide used for spreading the blood and one of the corners of the opposite end of the same slide is cut off, so that the length of the spreading edge is about 1·5 centimetres. (Fig. 2.)

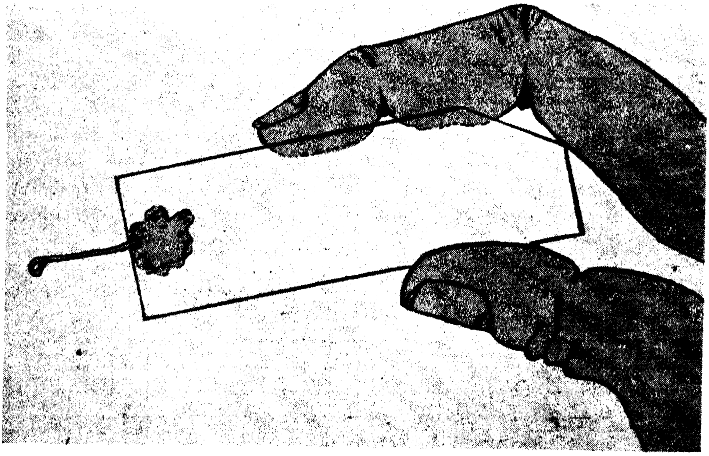

Figure 2.

The slide to which the loop is affixed is held at its edges with the thumb and forefinger and about midway between its ends, and a drop of blood is then taken up with the loop. After the drop has been deposited on to another slide held in the left hand, the slide carrying the platinum loop is simply tilted and with its narrow edge the blood is spread in the customary manner of pushing the spreader towards the other end of the under slide.

The process of first putting the loop down and taking up the slide for spreading the blood is thus eliminated and the smear is, therefore, prepared more quickly. In making smears from ostrich blood in which not only the thrombocytes but also the leucocytes clump very soon after the blood is drawn, a delay of some seconds in spreading the blood may vitiate the making of a smear satisfactory for differential counts.

By using a narrower spreader the blood is prevented from spreading over the long edges of the under slide and thus leucocyte counts can be satisfactorily made vertically across the smear, as the edges of the smear can be included in the fields examined. The size of the loop used for taking up the blood was always such that the length of the smear was about 3·5 cm. when the drop had been spread and as already indicated the breadth was about 1·5 cm. Dispensing with the use of the loop by taking up blood as it issues from the needle with the edge of the spreader proved unsatisfactory, for usually too much blood adhered to the edge with the result that the smears were too thick.

Staining of Smears.

May-Grunewald and Giemsa stain (as used by Pappenheim) Giemsa's, Leishman's, Jenner's and Wright's stain were all tried as recommended in text books and by individual workers. Wright's stain was finally adopted.

Staining with Wright's stain entails little work and the smear is usually ready for examination within ten minutes. This stain gave as good and uniform results as any of the others, and proved more satisfactory than some of them for the purpose of differentiating between the lymphocytes and the monocytes. The stain was prepared as prescribed by Carleton (1926) and used as recommended by Slider and Downey (1929).

In properly stained smears the cytoplasm of the red cells is a fawn colour and sometimes fine azurophil granules can be seen in the monocytes. With some samples of stain the diluted stain need only be left on the smear for 5 minutes whereas with others 10 minutes or more are necessary. Experience is the best guide as to how long to stain and to wash.

Pepper and Farley (1933) state: " The difficulty with methylene azur-eosin stains is to obtain the proper balance of eosin and basic stain. Muddy blue preparations are the result of too much alkali in the stain. To keep the stain at just the proper reaction is difficult."

For diluting Wright's stain McJunkin (1920) uses, instead of distilled water, a buffer solution made from monopotassium phosphate and dibasic sodium phosphate. This solution has a PH of 6·4 and will absorb excess alkali without change in the reaction of the solution.

The best results were usually obtained when Wright's stain was used immediately after the smears were prepared.

CELL MEASUREMENTS.

The cells were measured with a Leitz ocular micrometer that had been carefully calibrated, and their measurements are given under " Morphology of the Blood ". Ponder (1924) states that this method has been used with variations for the last seventy years and that nearly all tables of the average sizes of erythrocytes contain values determined in this way. He considers that at times results so obtained may have attached errors amounting to 10 per cent. and he remarks as follows: " When a body so small as the red cell is examined under the microscope owing to the fact that there is a limit of resolution to even the most perfect optical system the edge of the body is not seen distinctly but as a blurred band, the true edge being located somewhere in this " spurious disc " its position depending on the refractive indices of cell and surrounding fluid respectively. However carefully the system is adjusted a blurred band of at least 0·25μwill replace the cell edge and if no special precautions are taken the band may be as wide as 0·4μ."

There were no facilities for applying the photographic method described by Ponder (1934) and this author states in his work (dated 1924) that Pyper's (1919) diffraction method cannot be applied to avian red cells because of their oval shape.

MORPHOLOGY OF OSTRICH BLOOD.

The cells in the blood of the ostrich closely resemble the corresponding types of cells in the blood of the fowl, and they may be best considered by first reviewing the literature on the morphology of fowl blood.

Most investigators are agreed that the following types of cells are present in the normal blood of the fowl; red cells, thrombocytes and five varieties of white cells, viz., lymphocytes, monocytes, cells with polymorphus nuclei and spindle-shaped acidophil granules in their cytoplasm, cells with polymorphous nuclei and round acidophil granules in their cytoplasm, and basophiles.

Gibbs (1934) refers to a " polymorphonuclear leucocyte without rods and granules " as also being present in normal fowl blood in addition to the above-mentioned types of cells, while several workers saw no monocytes, and the findings of those who do record the presence of monocytes differ considerably. Thus, to quote only some of the workers listed in Table (2): —

Warthin (1907), Hedfeld (1911), Burchardt (1912) and Kleineberger and Carl (1912) found no monocytes in fowl blood. According to Wirth (1931), Steen (1913) is of opinion that they do occur, but only seldom, Salomon (1919) records 1 to 2 per cent., Fritsch (1920) 2 per cent. and Romer (1921) 1·4 per cent. Wirth (1931) records 2 per cent. On the other hand, Ellerman and Bang (1908) counted 23 per cent., Schmeisser (1915) 19·4 per cent., Breusch (1928) 9·2 per cent., Forkner (1929) (using supravital technique) 17·1 per cent. and Cook and Dearstyne (1934) (using supravital technique) 4 to 33 per cent.

These discrepancies serve to emphasise the difficulty of differentiating between lymphocytes and monocytes in ordinary stained preparations of fowl blood; in ostrich blood it is as difficult.

There can be no dispute about the presence of the elements which Sugiyama (no date) and others consider to be thrombocytes, but Blain (1928) states that he identified no structures corresponding to the platelets of mammals and he does not mention the thrombocytes.

Haematological terminology also gives rise to some confusion, entirely different cytological entities being called by the same name, or the same cytological entity by a variety of terms. Especially is this true of the cells with the spindle-shaped acidophil granules and those with the round acidophil granules, as will be observed from Magath's (1934) table given below.

Polymorphonuclear Leukocytes in Birds.

Author.	Date.	Eosinophilic rods.	Eosinophilic granules.
		Eosinophilic leukocytes.	
Bizzozero and Torre	1880	"Little spindleshaped bodies"	Granular forms.
		Eosinophilic leukocytes.	
Grunberg..........	1901	Crystalloid..............	Globular-like granules.
		Eosinophilic leukocytes.	
Cullen.............	1903	Crystalloid..............	Granular forms.
		Polymorphonuclear eosinophils.	
Warthin...........	1907	Crystalloid type..........	Granular type.
Goodall.....	1910	Polymorphonuclear leucocyte with rods	Eosinophil.
Kleineberger and Carl	1912	Pseudo-eosinophil.........	Eosinophil (with small granules)
		Polymorphonuclears.	
Schmeisser.........	1916	Eosinophilic rods.........	Eosinophilic granules.
Burnett............	1917	"Polymorphs"...........	"Eosins."
Fritsch............	1920	Pseudo-eosinophils........	Eosinophils.
Hayden............	1927	Polymorphonuclears.......	Eosinophils.
		Polymorphonuclears.	
Blain..............	1928	Eosinophilic rods.........	Eosinophilic granules.
Forkner...........	1929	Eosinophils..............	Pseudo-eosinophils.
		Polymorphonuclears.	
Thompsen and Engelbrethholm	1931	Pseudo-eosinophils........	Eosinophils.
Maximow and * Bloom	1931	Heterophil...............	Eosinophil.
Wirth*............	1934	Pseudo-eosinophiles.......	Eosinophiles.

* Investigators listed by the writer.

Burnett (1917) considers that the name "polymorphonuclear leucocyte with eosinophilic rods" is inexcusable. A "polymorphonuclear leucocyte" in his opinion signifies not merely a leucocyte with a polymorphous nucleus but the specific name of a certain kind of leucocyte, otherwise called "neutrophil".

Maximow and Bloom (1931) adopted the term "heterophil", first used by Kyes, as a comprehensive term to include the group of cells whose granules, though constant in a particular species, differ in form, size and staining reaction according to species. Hence the cell with the spindle-shaped granules was named "heterophil", and in avian haematology investigators have apparently applied this term only to that particular cell.

The names "heterophile" and "eosinophile" for the cells with the spindle-shaped granules and for those with the round granules respectively appear to the writer the most appropriate and they will be so used henceforth.

In smears prepared from ostrich blood and stained with Wright's stain the cells are differentiated by the following characters:

Erythrocytes.

Special interest attaches to the erythrocytes of the ostrich, for in smears of the blood of normal birds these cells may sometimes be seen in various stages of development. In this study detailed descriptions will be given only of the mature cells, the cells which are almost mature (polychrome erythrocytes) and those closest to the stem cell or haemocytoblast (basophil erythroblasts—Ferrata cited by Furth 1931).

Mature erythrocytes.—(Fig. 1 on plate.) These cells vary in shape from elliptical to almost circular, the younger forms tending to be round, but in smears many irregular shapes may occur owing to pressure. Occasionally a cell may be seen without a nucleus. The cytoplasm is homogeneous and as stated under "Staining of Smears" when correctly stained with Wright's stain it is a fawn colour.

The nucleus which is usually oval is an extremely pachychromatic and trachychromatic type. The basichromatin is arranged to form a coarse pattern in angular or roughly rounded blotches which frequently cause irregular elevations of the nuclear membrane and between which the oxychromatin forms a lighter meshwork. Very occasionally a mature cell may be seen with two nuclei or one in which the long axis of the nucleus is situated transversely to the long axis of the cell. The nucleus may also be small and pycnotic.

Two hundred regularly shaped and fully developed cells were measured at random in dried, stained smears and the length varied from 11·5μ to 15·93μ and the breadth from 7·08μ to 9·73μ, the average length being 13·98μ and the breadth 7·92μ. The measurements of the nuclei were as follows: Length 4·07μ–7·96μ, average length 6·33μ, breadth 1·77μ- 2·65μ, average breadth 2·19μ.

In practically every smear there may be seen roundish structures which are light purple in colour and irregular in outline. They are of loose structure and measure about 8μ in diameter. These are injured nuclei of ruptured erythrocytes (smudges). Sometimes part of the cytoplasmic rim enclosing a certain amount of cytoplasm similarly stained to the cytoplasm of the ripe erythrocyte may still be seen partly surrounding the body. These structures occur no matter in what way or how carefully smears are prepared.

In moist preparations the erythrocytes of the ostrich have a buff yellow colour and the nuclei appear indistinct against the colour of the haemoglobin. The nuclear structure is almost invisible but in a cell with little haemoglobin the oval shape and the clumps of chromatin of the nucleus can be seen. Cells with little haemoglobin cannot always be easily recognised in the counting chamber and they may be mistaken for free nuclei, thrombocytes or even small lymphocytes. Only by close observation and suitable manipulation of the condenser can the faint cell membrane be discerned.

Neser (1923) states with reference to equine erythrocytes: "In smears, however, the cells are more or less stretched out and the measurements are in consequence larger than those obtained in moist preparations", but Ponder (1934) remarks, "Although the point has been the subject of considerable controversy in the past I think it can fairly be said that it is now established that the red cell diameter is from 8–16 per cent. less when the cell is dried than when it is floating in plasma or serum". The writer also made measurements of ostrich red cells bathed in plasma, the following procedure being followed: Fresh heparinised blood was diluted a hundred times with its own plasma. The diluted blood was then introduced into a red cell counting chamber and the preparation ringed with vaseline. Thus the cells were not subjected to any pressure and the preparation remained suitable for a long time. Two hundred cells were measured and the dimensions were as follows: Length 15μ–16·5μ, average length 15·25μ, breadth 7·75μ–10·25μ, average breadth 9·25μ. From Table 1 it will be seen that these measurements approximate closely the figures given by Gulliver and by Hayem.

Polychrome Erythrocytes.—(Fig. 2 on plate.) The cells approaching maturity resemble closely in shape and size the fully developed cell. The cytoplasm shows only slight polychromasia, staining a light grey colour. The nuclei are larger and more oval than those of the mature cells and they show large angular clumps of chromatin which stain a slightly paler colour than the nuclear chromatin of the ripe cell.

Basophil Erythroblasts (Fig. 3 on plate). These cells are usually almost round but may be oval and they vary considerably in size. The larger forms usually measure about 12·4μ in diameter. The cytoplasm is very basophilic and the cytoplasmic layer around the nucleus is narrower than that of the polychrome erythrocyte. There may sometimes be seen in the cytoplasm granules which appear in staining reaction and size very similar to the azurophil granules of lymphocytes. The nuclei are as a rule circular and large and though the chromatin particles which stain a dark purple colour may be denser than in the more mature cells yet the tendency toward a checkerboard distribution of angular particles of chromatin is apparent.

Between the polychrome erythrocytes and the basophil erythroblasts may be seen various intermediate forms varying in shape, size and staining reaction. Some may be round and others oval. Round forms measuring only 7·9μ also occur. Immature forms are very occasionally seen in a state of mitosis and the cytoplasm of such cells is usually less basophilic than that of basophil erythroblasts. (Fig. 4 on plate.)

Reticulation can be favourably studied in the immature cells by staining them by the method used by Magath and Higgins (1934). The reticulations are heavy and look like a network in the very young cells, whereas in the older cells only a few strands or dots are to be seen.

The percentage of immature red cells which can be recognised in smears stained with Romanowsky stains was determined by counting, with the use of an Ehrlich eyepiece, 400 red cells. The results are shown in Tables 3-10 and a statistical analysis of them is given in Table 11.

The data were analysed statistically by the following formulae:—

(*a*) Standard deviation (σ)............ = $\sqrt{\dfrac{\Sigma d^2}{n-1}}$

(*b*) Standard error.................... = $\dfrac{\sigma}{\sqrt{n}}$

(*c*) Coefficient of variability............ = $\dfrac{\sigma\,100}{M}$

Σd^2 = Sum of the differences squared.

n = Number of observations.

M = Mean.

In the smears from the normal ostriches (1–5) these cells constituted 2·5±0·1 per cent. (Standard deviation 1·1; Coefficient of variability 47·2 per cent.) of the red cells, ranging from 0 per cent. to 7·0 per cent. It will be seen that the results from birds 6–17 closely agree with those from the normal birds, but it is noteworthy that only one immature erythrocyte was seen in the smears from the worm-infested birds 18–22.

The number of basophil erythroblasts per c.mm. was estimated by determining from the smear the ratio between these and the leucocytes and applying this ratio to the total leucocyte count. The ratio was computed from the number of basophil erythroblasts enumerated each time 200 leucocytes were counted.

TABLE 3.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (See pages 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocytes per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	PER CENT.				
			Mths.	*Days.*								L.	M.	H.	E.	B.
1	Male...	25/ 5/35	6	25	45·9	1,840,000	2·5	0	24·94	12,235	21,049	21·7	3·7	54·0	11·7	8·7
		17/ 6/35	7	17	45·8	1,944,000	2·0	0	23·69	13,860	25,301	23·0	2·2	55·7	10·5	8·5
		29/ 8/35	9	29	47	1,927,000	2·0	0	24·35	6,223	25,444	32·0	2·0	57·5	4·5	3·5
		25/ 9/35	10	25	47	1,910,000	4·0	0	24·60	11,869	28,672	24·2	2·7	60·0	5·0	8·0
		30/11/35	13	—	48	1,880,000	1·0	0	25·53	12,337	15,578	21·0	2·7	64·0	4·2	8·0
		30/ 1/36	15	—	50·2	1,950,000	1·5	0	25·74	7,526	21,344	21·2	3·5	58·2	10·7	6·2
		13/ 2/36	15	13	49·2	2,064,000	2·2	0	23·88	14,076	27,671	29·0	2·7	44·5	15·3	8·3
		15/ 4/36	17	15	50·6	1,970,000	2·0	0	25·68	6,136	20,408	32·5	3·7	48·5	8·0	7·2
		9/ 5/36	18	9	51·4	2,117,000	1·0	0	24·36	9,234	22,852	26·5	3·0	56·0	4·0	10·5
		20/ 7/36	20	20	51	1,920,000	3·0	146	26·56	12,264	29,255	23·7	8·5	58·7	2·7	6·2
		26/ 8/36	21	26	54	2,199,000	2·2	0	24·59	8,925	21,059	29·2	1·7	41·5	19·7	7·7
		22/ 9/36	22	22	52	2,000,000	2·0	0	26	12,190	22,966	23·2	5·0	58·5	3·4	9·7
		1/12/36	25	1	52	1,927,000	4·0	183	26·94	6,014	12,326	30·6	4·3	51·2	4·5	9·2
		4/ 2/37	27	4	52·4	1,967,000	4·7	258	26·59	8,944	17,253	27·7	3·2	52·7	6·5	9·7
		19/ 5/37	30	19	54	2,020,000	3·0	465	26·73	9,207	18,600	28·1	4·1	51·5	5·7	10·3

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

Table 4.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (See pages 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocytes per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P./R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
			Mths.	*Days.*								L.	M.	H.	E.	B.
2	Male...	9/ 7/35	8	9	44·4	1,720,000	2·5	246	25·81	12,160	24,751	27·0	2·5	60·0	8·7	1·7
		29/ 7/35	8	29	46	1,756,000	1·0	0	26·13	2,478	23,617	30·7	2·2	50·5	14·7	1·7
		25/ 9/35	10	25	44·4	1,729,000	2·2	0	25·66	8,554	18,251	27·0	4·7	58·2	8·2	1·7
		26/10/35	11	26	45	1,749,000	1·5	0	25·71	10,509	18,729	33·0	3·5	50·5	9·0	4·0
		7/12/35	13	7	43·8	1,653,000	1·5	0	26·54	10,608	15,543	32·5	4·5	48·2	9·5	5·2
		19/ 2/36	15	19	45·1	1,757,000	2·0	111	25·77	13,328	22,333	37·0	3·1	46·3	7·2	6·2
		15/ 4/36	17	15	43·8	1,700,000	1·7	0	25·76	9,660	23,103	34·5	2·5	52·0	4·5	6·5
		22/ 5/36	18	22	49	1,840,000	4·0	184	26·63	19,623	18,571	26·7	3·2	63·0	3·5	3·5
		14/ 7/36	20	14	49·1	1,830,000	1·0	0	26·77	7,020	21,567	22·2	3·0	60·0	9·7	5·0
		26/ 8/36	21	26	47·4	1,844,000	2·7	0	25·76	9,114	19,768	25·5	3·5	53·7	10·5	6·7
		26/ 9/36	22	26	46	1,983,000	1·0	0	23·23	13,746	17,461	25·0	3·0	62·5	4·7	4·7
		27/11/36	24	27	46·4	1,710,000	1·0	88	27·13	8,272	17,665	25·0	3·2	63·2	4·0	4·5
		21/12/36	25	21	47·1	1,831,000	2·2	318	25·73	11,554	21,312	25·5	6·0	59·2	6·0	3·2
		4/ 2/37	27	4	47·5	1,845,000	5·2	660	25·81	9,130	22,070	27·0	1·7	55·2	7·5	8·5
		19/ 5/37	30	19	50	1,903,000	2·2	195	26·31	11,088	13,157	25·5	6·0	59·2	6·0	3·2

* Abbreviations:—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 5.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (See pages 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
			Mths.	*Days.*								L.	M.	H.	E.	B.
3	Male...	12/ 7/35	8	12	48·9	1,916,000	1·2	156	24·45	9,984	31,303	26·7	2·5	60·7	8·2	1·7
		26/ 9/35	10	26	48	1,862,000	2·7	110	25·80	10,560	22,098	25·2	2·7	63·2	7·7	1·0
		9/12/35	13	9	46·5	1,806,000	2·2	200	25·69	14,600	20,044	26·5	3·7	59·0	7·7	3·0
		21/ 2/36	15	21	47	1,837,000	4·0	336	25·68	14,803	22,548	36·2	2·5	54·2	4·2	2·7
		15/ 4/36	17	15	45·4	1,873,000	2·5	0	24·27	14,060	29,596	26·5	3·2	65·5	3·0	1·7
		27/ 5/36	18	27	49·4	1,940,000	2·0	392	25·46	9,800	19,673	29·7	1·2	62·0	5·2	1·7
		14/ 7/36	20	14	52	1,920,000	3·0	488	27·08	10,248	24,495	28·7	1·7	59·0	7·2	3·2
		28/ 9/36	22	28	52	2,017,000	3·0	721	25·87	9,064	20,611	28·0	3·0	60·0	6·5	2·5
		27/11/36	24	27	48·5	1,980,000	3·0	206	24·49	5,562	20,618	28·5	3·5	55·2	8·7	4·0
		21/12/36	25	21	50·5	1,963,000	2·0	220	25·76	12,210	22,077	23·2	2·7	64·2	6·5	3·2
		4/ 2/37	27	4	50·5	1,962,000	4·0	792	25·76	10,593	19,994	28·5	3·2	57·2	7·0	4·0
		19/ 5/37	30	19	51	1,963,000	6·0	388	26·02	8,148	19,436	23·0	4·2	64·7	4·7	3·2

* Abbreviations:—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

Table 6.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (See pages 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	$\frac{\text{R.P.}}{\text{R.C.}}$	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
			Mths.	*Days.*								L.	M.	H.	E.	B.
4	Female.	21/ 5/35	6	21	45·4	1,693,000	3·0	0	26·86	20,086	24,296	27	2·7	56·2	12·7	1·2
		26/ 8/35	9	26	47	1,870,000	3·0	0	25·13	4,752	19,871	31	0·5	59·2	7·7	1·5
		24/ 9/35	10	24	48	1,750,000	3·2	0	27·42	6,018	20,459	27·5	2·7	62·5	4·7	2·5
		30/11/35	13	—	49·9	1,926,000	2·7	0	25·85	12,500	20,148	31	1·0	50·7	11·7	5·5
		27/ 1/36	14	27	47·9	1,948,000	2·5	0	24·56	17,191	23,496	31·5	2·1	46·5	14·6	5·1
		15/ 4/36	17	15	48·9	1,840,000	2·5	0	26·57	8,814	15,663	35·5	3·5	40·5	16·5	4·0
		9/ 5/36	18	9	49·9	1,930,000	0	0	25·85	9,625	15,325	30·5	2·7	52·2	8·0	6·5
		3/ 6/36	19	3	50	1,920,000	3·0	0	26·04	12,274	23,529	19·2	2·5	71·5	3·2	3·5
		20/ 7/36	20	20	47·8	1,740,000	2·5	161	27·47	16,422	32,273	25·2	5·5	61·7	6·0	1·5
		26/ 8/36	21	26	51	1,993,000	2·2	106	25·62	12,190	21,252	26·7	3·0	63·7	3·2	3·2
		22/ 9/36	22	22	51	1,890,000	4·2	140	26·98	15,194	14,157	25	2·7	64·7	2·0	5·5
		24/11/36	24	24	49	1,920,000	4·0	114	25·52	7,980	22,866	26·8	0·6	64·8	3·5	4·1
		19/12/36	25	19	49·6	1,926,000	2·7	71	25·84	10,008	14,399	30·5	1·5	59·2	3·2	5·5
		4/ 2/37	27	4	50·8	1,981,000	2·0	0	25·65	10,476	21,759	28·2	3·2	55·7	7·2	6·2
		17/ 5/37	30	17	50·6	1,900,000	3·5	0	26·63	12,852	21,514	27·7	3·0	60·5	4·7	4·0

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 7.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (See pages 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	PER CENT.				
			Mths.	*Days.*								L.	M.	H.	E.	B.
5	Female.	15/ 7/35	8	15	53	2,266,000	1·5	395	23·34	2,686	15,880	21·5	3·5	72·0	1·2	1·7
		28/ 9/35	10	28	47·9	1,980,000	3·7	116	24·19	10,374	23,329	21·2	2·5	74·5	0·7	1·0
		20/11/35	12	20	48	1,856,000	1·0	0	25·80	6,439	27,495	23·2	2·2	68·5	1·7	4·2
		21/ 1/36	14	21	40·6	1,676,000	3·0	0	25·94	8,613	19,959	17·8	2·5	75·5	1·0	3·1
		24/ 2/36	15	24	50	1,863,000	1·0	0	26·88	13,000	20,862	23·7	4·2	63·2	2·2	6·5
		15/ 4/36	17	15	45·7	1,853,000	1·0	0	24·70	4,646	20,164	17·0	2·0	78·5	0	2·5
		27/ 5/36	18	27	53·4	2,110,000	2·0	0	25·30	7,426	18,041	24·5	1·5	69·7	0·7	3·5
		14/ 7/36	20	14	53·8	2,096,000	2·2	182	25·61	15,168	19,204	25·5	1·5	65·7	1·7	5·5
		28/ 9/36	22	28	50·9	1,883,000	3·0	1,717	27·07	15,958	20,214	24·7	2·0	66·2	2·7	4·2
		24/11/36	24	24	53·5	1,853,000	7·0	6,283	28·91	7,072	20,733	33·5	3·7	53·0	3·2	6·5
		12/12/36	25	21	54	2,195,000	3·0	1,339	25·54	9,476	20,704	23·7	3·0	68·5	2·0	2·7
		4/ 2/37	27	21	53·2	2,167,000	1·2	890	25·64	12,905	17,890	26·5	2·8	62·0	3·6	5·0
		15/ 5/37	30	15	52	1,953,000	3·0	89	26·66	8,811	17,857	18	3·5	68·7	3·7	6·0

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 8.

Blood Cell Counts, etc., of Ostrich with Club Foot. (See pages 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocytes per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Baso-phil-erythroblasts per c.mm.	$\frac{R.P.}{R.C.}$	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
			Mths.	*Days.*								L.	M.	H.	E.	B.
6	Female.	21/ 7/35	8	21	47	2,053,000	3·0	524	22·92	4,061	26,210	28·2	1·5	66·0	3·2	1·0
		10/ 8/35	9	10	49	1,921,000	2·2	296	25·52	12,580	29,668	28·2	3·2	62·5	4·0	2·0
		25/ 9/35	10	25	43	1,669,000	3·0	219	25·74	14,235	43,914	21·7	4·5	67·5	4·5	1·7
		20/11/35	12	20	51	2,010,000	1·2	452	25·37	9,266	45,372	25·7	3·0	64·7	3·2	3·2
		24/ 2/36	15	24	49	1,943,000	1·5	290	25·25	18,415	29,039	26·2	3·2	65·7	0·5	4·2
		15/ 4/36	17	15	48·2	2,023,000	1·5	0	23·86	12,862	23,598	21	2·2	71·5	0·7	4·5
		20/ 5/36	19	—	46·9	1,863,000	2·0	0	25·21	26,650	40,986	14·2	6·0	78·5	0·2	1·0
		20/ 7/36	20	20	48	2,033,000	3·0	595	23·64	10,948	23,842	26·7	6·2	61·0	3·7	2·2
		7/ 9/36	22	7	52·3	2,034,000	2·5	364	25·76	19,838	36,463	15·7	0·7	80·0	0·5	3·0
		28/ 9/36	22	28	52·8	2,053,000	1·7	0	25·75	15,480	25,808	20	3·7	73·7	0·5	2·0
		1/12/36	25	1	50·1	1,913,000	2·2	192	26·23	5,238	19,323	14·6	2·5	81·0	0·3	1·5
		23/12/36	25	23	50·5	1,961,000	1·7	0	25·76	10,956	26,510	16	4·2	77·7	0·2	1·7
		9/ 2/37	27	9	50	1,940,000	2·5	0	25·77	6,400	25,623	15·5	4·7	75·0	1·5	3·2
		17/ 5/37	30	17	49	1,970,000	2·5	87	24·87	7,221	17,422	25·5	1·7	70·5	0·7	1·5

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 9.

Blood Cell Counts, etc., of clinically healthy Ostriches on which Post-Mortem Examinations were not conducted. Kept on Farm Nagwag, Bredasdorp District. (See page 429.)

No.	Sex.	Date Bled.	Age.	R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	PER CENT.				
											L.	M.	H.	E.	B.
7	Male...	1/ 5/36	Over 3 years	38·4	1,403,000	3·2	0	27·42	12,444	12,239	14·2	4·0	73·0	6·0	2·7
8	,,	,,	,, ,,	47·4	1,790,000	0	0	26·48	8,946	8,409	11·7	1·0	69·0	14·2	4·0
9	,,	,,	,, ,,	40·4	1,580,000	1·0	0	25·56	17,415	16,146	15·7	3·0	62·0	18·0	1·2
10	,,	,,	,, ,,	41·7	1,510,000	3·0	330	27·61	13,002	13,271	8·7	3·2	68·2	15·2	4·5
11	,,	,,	,, ,,	42·3	1,553,000	0·5	77	27·29	13,650	15,560	14·0	4·5	69·7	9·0	2·7
12	Female.	,,	,, ,,	41·4	1,563,000	3·0	0	26·53	12,300	24,570	5·2	2·0	73·5	18·7	0·5
13	,,	,,	,, ,,	42·3	1,620,000	1·7	0	26·11	12,118	14,336	12·2	4·0	69·0	9·5	5·2
14	,,	,,	,, ,,	41·5	1,533,000	1·5	0	27·12	7,720	7,868	13·7	4·2	68·7	3·2	10·0
15	,,	,,	,, ,,	41·4	1,637,000	0·7	0	25·24	15,330	14,122	8·0	3·0	79·5	7·2	2·2
16	,,	,,	,, ,,	49·4	1,790,000	2·0	0	27·59	13,536	9,347	4·0	2·0	83·2	9·5	1·2
17	,,	,,	,, ,,	42·4	1,430,000	4·0	70	29·65	15,336	14,154	6·7	4·5	72·7	12·5	3·5

* Abbreviations:—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 10.

Blood Cell Counts, etc., of unthrifty Ostrich Chicks which on Post-Mortem Examination showed marked Verminosis. From Farm Vanderstelskraal, Bredasdorp District. (See page 429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocytes per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	$\frac{\text{R.P.}}{\text{R.C.}}$	Thrombocytes per c.mm.	Leucocytes per c.mm.	PER CENT.				
			Mths.	*Days.*								L.	M.	H.	E.	B.
18	Female.	15/ 3/36	4	—	34·3	1,400,000	0	0	24·50	11,256	16,973	26·2	3·0	54·0	13·7	3·0
		2/ 4/36	4	17	29·4	1,173,000	0	0	25·12	4,650	30,033	7·2	2·7	89·7	0·2	0
19	”	16/ 3/36	4	—	29·5	1,330,000	0	0	22·18	14,112	22,561	45·7	2·0	48·2	1·5	2·5
20	Male...	16/ 3/36	4	—	38·0	1,573,000	0	0	24·20	7,392	11,204	26·0	3·5	68·2	1·0	1·2
21	”	20/ 3/36	4	—	25·3	806,000	0	43	31·23	15,394	8,697	27·5	6·2	47·5	8·2	0·5
22	”	16/12/36	2	—	41·8	1,707,000	1	204	24·44	14,008	13,793	15·5	9·5	70·7	2·2	2·0

* Abbreviations:—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 11.

Statistical Analysis of Counts of Immature Erythrocytes.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1.................	Male.......................	15	1·0	4·7	2·4	0·2	1·0	41·6
2.................	Male.......................	15	1·0	4·0	2·1	0·2	1·1	50·0
3.................	Male.......................	12	1·2	6·0	2·9	0·3	1·2	41·3
4.................	Female.....................	15	0	4·2	2·7	0·1	0·6	22·2
5.................	Female.....................	13	1·0	7·0	2·5	0·4	1·6	64·0
1, 2 and 3.........	Males.......................	42	1·0	6·0	2·5	0·1	1·1	44·0
4 and 5...........	Females...................	28	0	7·0	2·6	0·2	1·1	42·3
1 to 5.............	Males and Females.........	70	0	7·0	2·5	0·1	1·1	44·0
6.................	Female.....................	14	1·2	3·0	2·1	0·1	0·6	28·5
7 to 11...........	Males.......................	5	0	3·2	1·5	0·5	1·3	86·6
12 to 17...........	Females....................	6	0·7	4·0	2·1	0·4	1·0	47·6
7 to 17...........	Males and Females	11	0	4·0	1·8	0·3	1·0	55·5
18 to 22..........	Males and Females.........	—	0	1·0	0·1	—	—	—

* For particulars of birds, see pages 428–429.

The number of erythroblasts observed in smears per 200 leucocytes counted vary for the normal ostriches from 0 to 61 averaging 2·6, and the calculated total counts, which are listed in Tables 3–10, range from 0 to 6283, averaging 264 per c.mm. These cells constitute from 0 to 0·33 per cent. of the erythrocytes, averaging 0·013 per cent.

The maximum count of 6283 is exceptionally high, considering that it is over three times as high as the count nearest to it. It is not clear why bird No. 5 showed such a high count on 24/11/36 as no cause for unusual erythrocyte regeneration was evident. The bird always seemed healthy and only 5 c.c. of blood were drawn from it about two months previously. However, the counts from this bird during the period 28/9/36–4/2/37, are suggestive of unusual erythropoiesis.

The blood of ostriches Nos. 7–17 contained comparatively few basophil erythroblasts.

Thrombocytes. (Figure 5 on Plate.)

These cells, which in fowl blood have been named hematoblasts by Hayem (1879) and by Goodall (1910), are usually oval, nucleated and very unevenly distributed on the slide. They occur in pairs or groups of three or more, but are often seen singly. They measure usually about 8·8μ in length and 5·3μ in breadth. Occasionally a thrombocyte almost twice the normal size may be seen.

The nucleus is oval and usually occupies about three-quarters of the length and nearly the entire width of the cell. It is usually situated in the central part of the cell and is very basophilic, the basichromatin being arranged to form a coarse pattern.

The cytoplasm stains a pale grey and ordinarily there may be one or more acidophil granules. These are usually near the poles of the nucleus. The cytoplasmic rim is not always well marked. In films not prepared immediately after the vessel has been punctured the thrombocytes can be seen in masses in which it is difficult to distinguish the outline of individual cells. Both the cytoplasm and the nucleus become less distinct, the cytoplasm losing its structure first. Finally the cells appear merely as a mass of darkly stained roundish bodies. When thrombocytes have become thus transformed they may be mistaken by small lymphocytes but the cells can be differentiated by the following features: The outline of the lymphocyte, which is usually completely circular, is well marked, whereas that of the thrombocyte is very irregular and usually the cytoplasm of the lymphocytes does not appear to surround the nucleus completely.

In the counting chamber the thrombocytes when stained with Wiseman's solution, about which particulars are given under "Leucocyte Counts", look mottled and the red granules in the cytoplasm can usually be seen. No attempt, however, was made to count the thrombocytes in the counting chamber, for it was felt that the criteria by which these cells may be discriminated from free nuclei and small lymphocytes are not prominent enough to make accurate counting possible.

Lymphocytes. (Figs. 6 and 7 on plate.)

These cells are spherical and vary in size from 5·3μ to 14·1μ. They may be arbitrarily classed as small, intermediate and large lymphocytes, though the cells apparently do not belong to separate families but to one series. This was the conclusion arrived at by Magath and Higgins (1934) who, in order to test the validity of the theory that there are classes of lymphocytes based on size, measured the diameters of 100 lymphocytes from each of eight ducks, and plotted curves.

In the smaller cells the nucleus is usually round, but it may be oval or slightly indented and it may be situated centrally or eccentrically. It usually consists of heavy blotches of chromatin but sometimes its structure is almost homogeneous. The nucleus is stained purple. It occupies by far the greater part of the cell and in the smallest cells the cytoplasm is scarcely seen. The cytoplasm is pale blue and usually takes the stain better at the periphery than towards the nucleus, with the result that there is a sort of " halo " near the nucleus. The cytoplasm forms only a very narrow layer and usually does not appear to surround the nucleus completely. A varying number of azurophil granules sometimes occur in the cytoplasm and occasionally lingulate processes extend from the cytoplasmic rim. As already stated it may be very difficult to differentiate between very small lymphocytes showing very little or no cytoplasm and thrombocytes which have lost their usual structure.

In the larger cells the proportion of cytoplasm to nucleus is much greater than in the small cells and the cytoplasmic colour varies from a pale blue to a distinct blue. Otherwise the cells are identical with the small forms. The large cells often have irregular shapes in smears on account of being wedged in between the erythrocytes. (1)

Monocytes. (Figs. 8 and 9 on plate.)

The cells, which may be considered homologous with the mammalian monocytes, vary in form from round to slightly oval and measure from 8·8μ to 12·4μ in diameter but in the oval forms the long axis may measure up to 21·2μ.

The nucleus shows a loosely woven chromatin network, the chromatin being finely divided, and it is usually stained a paler purple than the nucleus of the lymphocyte. It may be nearly round, slightly indented, kidney-shaped or of irregular shape. The kidney-shaped nuclei are rare, however, and are usually seen only in the smaller cells. The nucleus usually occupies about half the cell in the larger forms, whereas in the small cells it may form three-quarters or more of the cell. It is usually eccentric and usually the cytoplasm does not appear to surround it completely.

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(1) Although the blood forming organs are not being considered in the present work yet it may be mentioned that no lymphatic glands could be found in the ostrich.

The cytoplasm has a greyish-blue colour and is more granular than that of the lymphocyte, having the ground-glass appearance usually seen in the monocytes of man. Sometimes the cytoplasm is dusted with innumerable minute azurophil granules. Small lingulate runners sometimes extend from the cytoplasmic rim.

These cells can be studied best in thin smears prepared very quickly and immediately after the blood has left the vessel. Otherwise many damaged forms may be seen.

Heterophiles. (Figs. 10 and 11 on plate.)

These cells are spherical and measure from 8·9μ to 17·7μ in diameter, but like the large lymphocytes they are often irregularly shaped through being wedged between the erythrocytes. The nucleus which stains a reddish violet is polymorphous with varying degrees of lobulation. Sometimes the nucleus consists of two or three lobes connected either by a thin or a thick strand of chromatin and usually the lobes are more or less oval. In ordinary stained preparations the lobes may sometimes appear to be quite apart, but when smears are stained with the " Analine-water thionin solution " as recommended by Pepper and Farley (1933) the connection, which is sometimes only a very fine thread of chromatin, can be easily seen.

The cytoplasm which is colourless is filled with large spindle and rod-shaped granules which measure about 1·75μ in length and stain a dull red. In the young cells the granules are roundish. On a few occasions peculiar staining effects were observed. The granules of practically all the heterophiles became transformed into small round granules, as seen in figure 12 on the plate, whereas the granules of the eosinophiles looked the same as usual. In other smears prepared at the same time from the same bird, the granules were as ordinarily seen. Many of the heterophilic granules were also roundish when vital staining was carried out either by the method described by Kolmer and Boerner (1931) or by that used by Magath and Higgins (1934) for the purpose of demonstrating reticulocytes.

Olson (1937) apparently observed similar results for in referring to the heterophilic granules of the fowl he remarks: " Frequently in routinely stained smears these cytoplasmic bodies are distorted and they may then be variable in shape ".

These phenomena are of especial interest, for Lundquist and Hedlund (1925) express the opinion that the heterophilic granules of the fowl are naturally round, only becoming spindle-shaped as the result of fixation and staining. According to these writers the heterophiles and the cells with the round acidophil granules (eosinophiles) are identical—the eosinophiles being the forms in which the granules retain their round shape.

Ellerman (1921) considers the two cell types distinct from each other as the heterophiles do not react to Graham's oxidase test, whereas the eosinophiles do. The writer can confirm this observation by Ellerman and has ascertained that in the blood of the fowl and the ostrich the eosinophiles are the only cells that are stained by Graham's benzidine stain.

Jackson (1936) in discussing myeloid leucosis and myelocytomas of fowls states that it seems usually to be assumed that the myelocytes present are the neoplastic counterparts of true eosinophile myelocytes because their granules are rounded, but that they are the precursors of the heterophiles for which he uses the name "pseudoeosinophiles". He further remarks: "The confusion arises because of the failure to realise that in the earlier myelocytic stages the pseudoeosinophilic granules are rounded, only becoming rod-shaped as the cell matures. Such rounded pseudoeosiniphilic granulations can be distinquished because the former (i) are coarser (ii) some of them are actually basophilic in reaction, developing eosinophilia at a later stage, and (iii) because on close examination a tendency can often already be seen to a slight elongation of the granules. For those who have difficulty with this conception it is essential to undertake a study of normal avian red marrow, when the above features will readily be determined; it must be borne in mind, however, that in the marrow true eosinophile myelocytes for comparison are often difficult to find and must be searched for".

The transformation of haemocytoblasts into heterophilic and eosinophilic myelocytes may be favourably studied in the ostrich for in its red marrow (¹) both types of myelocytes are found without difficulty.

A few heterophile myelocytes were once seen in a smear prepared from the blood of a normal ostrich—No. 5. The cells were round and measured about 17μ in diameter. They were completely filled with round coarse granules, some of which were basophilic in reaction. The nuclei were so obscured by the granules that the structure could not be judged. However, in the marrow it will be observed that the nuclei of these myelocytes are either round or oval. They are eccentric and stain purple. The acidophilic granules of the heterophile myelocytes stain a dull red and neither the acidophilic nor the basophilic granules of these cells stain with Graham's benzidine stain.

Eosinophiles. (Fig. 13 on plate.)

These cells are also round and measure from 8·7μ to 15·7μ. They are, therefore, of about the same size as the heterophiles.

The nucleus which stains a reddish violet may be a single polymorphous structure or it may consist of two lobes connected by either a slender or a thick strand of chromatin. Sometimes the lobes appear to be entirely separate. Usually the nucleus is bilobed. The cytoplasm which is colourless contains discrete round granules whose size and staining are uniform. They stain salmon red in marked contrast to the dull red colour of the granules of the heterophiles and they are also much smaller than the granules of the heterophiles. These granules stain a greenish yellow with Graham's benzidine stain.

Basophiles. (Figs. 14 to 15 on plate.)

The basophiles measure from 8·8μ to 10·6μ in diameter. The cell has a simple round nucleus which stains purple and shows a

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(¹) (*a*) The marrow examined was taken from the upper extremity of the tibia.
(*b*) It was observed that the femur of the ostrich is devoid of marrow.

diffuse chromatin arrangement. It is usually situated to one side of the cell and the cytoplasm does not appear to surround it completely. The cytoplasm contains large granules which stain a very dark purple colour, and there is considerable variation in the degree of concentration of the granules. Occasionally some of these may be seen scattered over the nucleus. These granules appear to be readily dissolved or washed out in the process of staining—for very often no granules will be seen—the cytoplasm which stains a faint blue presenting a reticular appearance. However, granules which are present in the cells after staining has been completed cannot be removed by washing the smears in water. Basophiles without granules can, nevertheless, be recognised by the round eccentric nuclei and the reticular appearance of the cytoplasm. (Fig. 16 on plate.) The granules of the basophile do not react to Graham's oxydase test.

Erythrocyte Counts.

Wiseman's solution was used as diluting fluid for the enumeration of both the red cells and the white cells. Further information about the technique employed in counting the red cells is given under "Leucocyte Counts" where it can be more conveniently considered.

Magath and Higgins (1934) state in their work on duck blood—"Counts of erythrocytes were made after the blood had been diluted with Hayem's solution to 200. A photographic method not yet reported was used; its accuracy is extremely high".

These workers used Wiseman's solution as a diluting fluid in enumerating the leucocytes, but for the erythrocyte counts Hayem's solution was used because the red colour of Wiseman's solution interfered with the photographic process of counting.(¹) This photographic method has been described by Berkson, Magath and Hurn (1935).

Elsdon-Dew (1937) describes an electrical apparatus for the performance of blood counts and mentions several advantages which this device has over the routine method.

Detailed data pertaining to the erythrocyte counts of twenty-two ostriches are shown in Tables 3–10, and in Table 12 a statistical analysis of the erythrocyte counts is given.

As stated on page 429 counts were made at intervals from each of the birds kept at Mariendahl, and the mean erythrocyte count of all the counts made during the period of about two years from the five normal birds (1-5) was 1,894,000 ± 14,939 (Standard deviation 124,000; Coefficient of variability 6·5 per cent.). The range was 1,653,000 to 2,266,000. The counts from bird No. 5 gave a comparatively high coefficient of variability which, however, would have been appreciably less but for the low result shown by this bird on 21/1/36.

It is not apparent why this result deviated so much from the others. The bird appeared healthy. Its blood showed no evidence of

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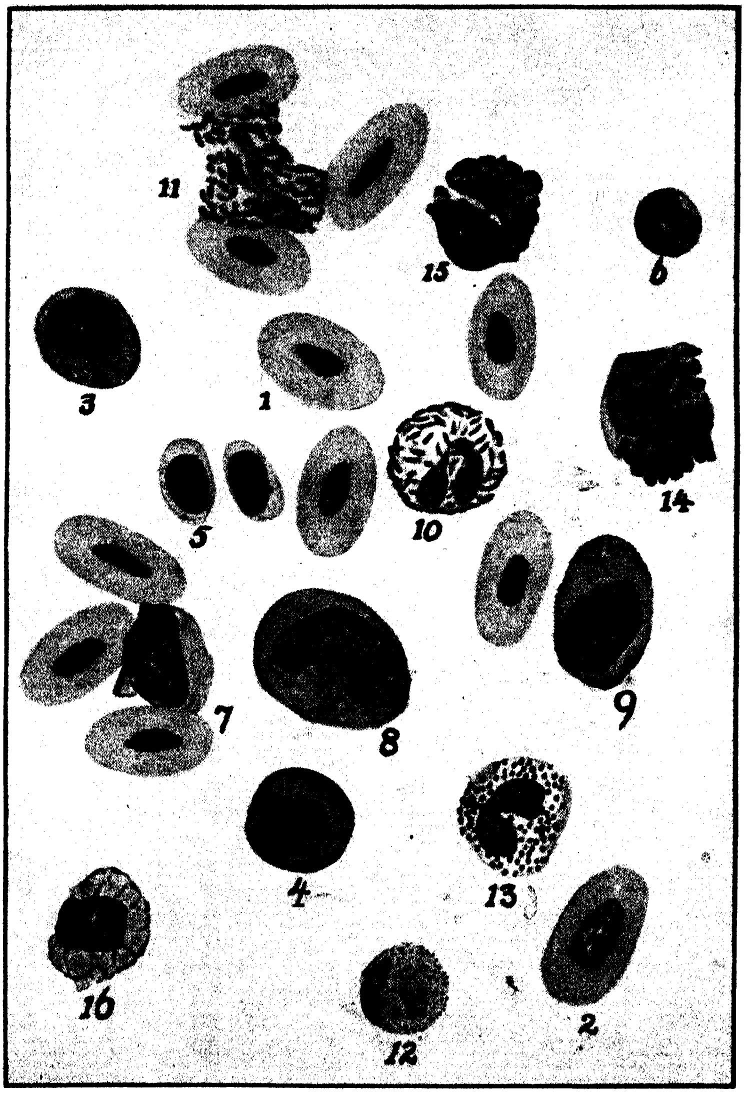
(¹) Personal communication from Dr. T. B. Magath.

BLOOD CELLS.



haemolysis and the accuracy of the result can be vouched for. It will be seen that the red cell counts were not significantly affected by the age or the sex of the birds.

The results from bird No. 6 also show a low coefficient of variability and the average count of this bird does not differ much from the mean figure obtained for birds numbered 1 to 5.

The results from the majority of the clinically healthy birds 7–17 are low compared with those from birds 1-5, and the difference between the averages of the two groups is statistically significant. As previously stated the faeces of some of these birds were examined and these contained many worm eggs. The low counts may, therefore, be possibly correlated with verminosis as in the case of the worm-infested birds, 18–22, which showed marked oligocythemia.

The figures recorded by Malassez (1872), Hayem (1879, 1889) and by Venzlaff (1911) (Table 1) differ appreciably from the average result shown by birds 1 to 5. But the results given by Malassez and by Hayem closely approximate the mean count obtained for birds 7 to 17.

Examination of Table 2 shows that the average counts reported for the fowl are considerably higher than those obtained for the ostrich. Available results for other birds also greatly exceed those for the ostrich.

Relative Volume of Corpuscles and of Plasma.

An electric centrifuge was used for the purpose of precipitating the red cells, and the precipitation tubes (Zeiss) were 12 cms. long and 0·6 cm. in diameter. As the centrifuge tubes were much wider than the precipitation tubes, the latter were fitted vertically into the centrifuge tubes in this way: In the centrifuge tube two corks were fitted, one at the bottom and one at the mouth. A precipitation tube was fitted into the centre of each cork through a hole just big enough for it. The tubes were all tested before use, and two tubes were used for each sample so as to check the one with the other.

The arm length of the centrifuge was 19 cm. when measured to the bottom of a centrifuge tube, and, at a top speed of 2,800 revolutions per minute, a limiting value of the red cell volume of ostrich blood was obtained in from 45 minutes to one hour. However, the centrifuge was always allowed to run for at least one and a half hours to make quite sure of the final reading at that speed. On many occasions the centrifuge was run at speeds lower than 2,800 r.p.m. so that results obtained at slower speeds could be compared with those given at top speed. The tubes were spun for 45 minutes and the results read. Centrifuging was repeated for another half hour and the readings again noted. This procedure was carried out until two successive readings had the same value. At 1700 r.p.m. the same readings were obtained as at top speed of 2,800 r.p.m. and the results at the lowest speed of 600 r.p.m. differed very little from those shown at the highest speed. But at the lowest speed a period of 5 to 6 hours was usually required before limiting values were obtained. Six typical results are recorded. Samples numbers 1, 2 and 3 were whole blood and 4, 5 and 6 heparinised blood.

Table 12.

Statistical Analysis of Erythrocyte counts.

Bird Number.*	Sex.	Number of Counts.	Maximum Count per c.mm.	Minimum Count per c.mm.	Mean Count per c.mm.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	2,064,000	1,840,000	1,976,000	24,090	93,230	4·7
2	Male	15	1,903,000	1,653,000	1,790,000	22,435	86,827	4·8
3	Male	12	2,017,000	1,806,000	1,920,000	18,349	63,490	3·3
4	Female	15	1,993,000	1,693,000	1,882,000	23,160	89,632	4·7
5	Female	13	2,266,000	1,676,000	1,981,000	48,055	173,000	8·7
1, 2 and 3	Males	42	2,064,000	1,653,000	1,893,000	17,746	115,000	6·0
4 and 5	Females	28	2,266,000	1,676,000	1,928,000	26,654	141,000	7·3
1 to 5	Males and Females	70	2,266,000	1,653,000	1,894,000	14,939	124,000	6·5
6	Female	14	2,053,000	1,669,000	1,956,000	27,005	101,000	5·1
7 to 11	Males	5	1,790,000	1,403,000	1,567,000	64,545	142,000	9·0
12 to 17	Females	6	1,790,000	1,430,000	1,595,000	50,000	120,000	7·5
7 to 17	Males and Females	11	1,790,000	1,403,000	1,583,000	37,575	124,000	7·8
18 to 22	Males and Females	6	1,707,000	806,000	1,331,000	132,500	318,000	23·8

* For particulars of birds, see pages 428-429.

Sample Numbers and Percentage Volume Readings.

Speed in R.P.M.	1.	2.	3.	4.	5.	6.
600.............	54	49·5	48·8	49	54	48
1,700............	53·6	49	48·5	48·9	53·5	47·5
2,800............	53·4	49	48·5	48·9	53·5	47·5

Neser (1923), using horse blood, found that the results obtained in 15 minutes were the same whether the centrifuge ran at 250 or 3,000 revolutions per minute, and he remarked: " It is not contended that very high speeds may not result in a closer packing of the red corpuscles or that very low centrifuge speeds will give rise to the same degree of packing as very high speeds. But the results point to this fact that, within certain limits of speed, the volume of the red corpuscles is in no way influenced by the speed. The readings obtained by the centrifuge are reliable and in no way dependent upon the speed of the centrifuge for all moderate speeds."

Millar (1925a) by centrifuging human blood at 4,350 r.p.m. obtained a final reading of 39·0. At 5,700 r.p.m. the final result was 37·1 and at 11,600 r.p.m. 35·0. Ponder (1934), using rabbit's blood, obtained a final result of 31·5 at 1,700 r.p.m. and at 14,000 r.p.m. the reading was 29·5. He remarks: " The attainment of ' constant volume ' consequently cannot be used as a test for the correct percentage volume having been reached unless we know which speed of the haematocrite produces neither incomplete packing nor compression of the cells, i.e., unless we solve a second problem very similar to the one whose solution is being sought. The speed of 4,000 r.p.m is supposed to be such a speed, but why this particular rate is selected I have not been able to discover. There is certainly no evidence that it is as ideal as it is thought to be." The observations by Millar and Ponder stress the importance, when recording percentage volume results, of stating both the arm-length of the centrifuge and the speed at which it rotated. This is, unfortunately, not often done. Facilities did not exist for testing the effects on the corpuscles of ostrich blood of speeds higher than 2,800 r.p.m., but from repeated observations made it may be stated with confidence that with a centrifuge as specified the same final results may be expected within a speed range of 1,700 to 2,800 r.p.m.

Special care was always taken to shake the blood in the bottle thoroughly before it was drawn off with the pipette for the purpose of determining the percentage volume and to fill the tubes exactly to the 100 mark. The blood was also run from the pipette into the percentage volume tubes with as little delay as possible to prevent sedimentation in the lower part of the pipette. Nevertheless there were sometimes differences in the readings of samples from the same bottle, although the maximum difference never exceeded 1·5.

The recorded percentage volume readings include the white cell volume, for this never once exceeded more than 2 per cent. of the blood volume of fresh blood and often constituted even less than 0·5 per cent. Often also it was impossible to observe a well defined margin between the red cell column and the white cell layer.

Neser (1923) made tests to ascertain in how far the centrifuge readings could be relied upon. For this purpose a large quantity of blood was drawn and citrated and this citrated blood was then centrifuged at definite intervals until there was evidence of haemolysis. He found that with horse blood the results remained constant for 36 hours and that the percentage volume decreased as soon as any sign of haemolysis appeared. The writer made similar tests with ostrich blood using heparinised, citrated, and also whole blood. The blood was kept in well stoppered bottles and successive fillings of the precipitation tubes were always from the same sample. Most of the samples were kept at room temperature, which varied from 15° C. to 25° C. according to the time of the year, as the tests were conducted at different times. The other samples, when not required, were kept in cold storage. The results are given in Table (13) and it will be observed that contrary to the findings of Neser (1923), who used horse blood, the percentage volume readings of heparinised, citrated, and also whole ostrich blood invariably increase with age and the older the sample the greater the increase. For example, this increase averaged 9·6 in the heparinsed samples five days old and kept at room temperature; and the average increase in the five day old heparinised samples kept in cold storage was 6·7. Haemolysis was usually well marked in samples a few days old. It would appear that the plasma of ostrich blood becomes so viscid after a time as to prevent such close packing of the cells as results when the plasma is fresh. This problem is being further investigated. Attempts were made to obtain more results with whole blood kept in cold storage but the blood usually coagulated within 24 hours.

It was noticed that in old samples the increase in volume was particularly marked in the white cell volume. For example, in sample number 4, which is typical, the white cell layer constituted only 1 per cent. of the blood volume when the blood was fresh, but it formed 6·9 per cent. of the blood volume when the cells were precipitated again five days later.

Although the results show that significant increases in the readings may not be expected even when the blood is 11 hours old, yet in these studies the precipitation of the blood from ostriches 1–6 and 18–22 was always completed within 3½ hours from the time that the blood was collected. The precipitation of the blood samples from birds 7–17 was completed within 6 hours after they were collected, for they had first to be conveyed a distance of over 100 miles.

As already stated under the heading " Preventing Coagulation of the Blood ", 0·5 c.c. of a 20 per cent. lithium citrate solution to 199·5 c.c. of blood was at first used as anticoagulant but this was later replaced by heparin—1 mg. powdered heparin to 5 c.c. blood. As the lithium citrate rendered the plasma slightly hypertonic and as the blood was also slightly diluted, observations were made to

	Sample No.	Temperature.	Anti a	s obtained.			
				5 Days.	6 Days.	15 Days.	30 Days.
	1	15° C.–25° C.....	Hepari	—	—	—	—
	2	"	"	—	—	—	—
	3	"	"	—	—	—	—
	4	"	"	56	—	—	—
	5	"	"	58·6	—	—	
	6	"	"	—	—	—	—
	7	"	"	—	—	—	—
	8	"	"	63	—	—	—
	9	"	"	60	—	—	—
	10	"	"	60·6	—	—	—
	11	"	"	—	—	—	—
	12	"	"	—	—	—	—
	13	"	"	—	—	—	—
	14	"	"	57	—	—	—
Average Increase..	—	—		9·6	—	—	—
	15	Kept in ice chest	Hepar	55·3	—	—	—
	16	" "	"	57	—	—	—
	17	" "	"	54	—	—	—
	18	" "	"	55	—	—	68
	19	" "	"	54·1	—	—	66
	20	" "	"	56·2	—	67·5	—
Average Increase..	—	—		6·7	—	—	—
	21	15° C.–25° C.....	Lithiu	—	—	—	—
	22	"		—	—	—	—
	23	"		—	—	—	—
	24	"		—	—	—	—
	25	"		—	—	—	—
	26	"		—	—	—	—
	27	"		—	—	—	—
	28	"		—	—	—	—
	29	"		—	—	—	—
	30	"		53·3	—	—	—
	31	"		58	—	—	—
	32	"		59·1	—	—	—
	33	"		—	—	—	—
	34	"		—	—	—	—
	35	"		—	—	—	—
	36	"		—	—	—	—
	37	"		—	—	—	—
	38	"		—	—	—	—
	39	"		—	—	—	—
	40	"		—	—	—	—
	41	15° C.–25° C.....	No an	—	—	—	—
	42	"	"	—	—	—	—
	43	"	"	—	—	—	—
	44	"	"	—	—	—	—
	45	"	"	—	—	—	—
	46	"	"	—	—	—	—
	47	"	"	54	—	—	—
	48	"	"	—	—	—	—
	49	"	"	—	—	—	—
	50	"	"	—	—	—	—
	51	"	"	—	—	—	—
	52	Kept in ice chest		—	—	—	—
	53	" "		—	—	—	—
	54	" "		—	—	—	—

ascertain to what extent the percentage volume readings of citrated blood differed from those of whole blood. On thirty-five occasions whole blood was collected and centrifuged along with blood collected immediately afterwards in a bottle containing lithium citrate solution. In no case did the reading of the whole blood vary from that of the citrated blood by more than 2 and as will be noted from Table 14 the average difference was only 1·36. A reading, very near to that of whole blood, was therefore obtained by adding 1·4 to the percentage volume reading of citrated blood and in this work all percentage volume readings of citrated blood were corrected accordingly.

TABLE 14.

Number of Sample.	Percentage Volume (Whole Blood).	Percentage Volume (Citrated Blood).	Difference.
1	47	45	2
2	48	46·5	1·5
3	49·3	48	1·3
4	50	49·2	0·8
5	47·8	46	1·8
6	49	48	1
7	46·9	45	1·9
8	51	49·5	1·5
9	48	46	2
10	45·2	44·3	0·9
11	45·7	44·3	1·4
12	50	48	2
13	49	47·2	1·8
14	46	46	0
15	49·2	47·9	1·3
16	49·3	47·6	1·7
17	50·2	48·4	1·8
18	50	48·5	1·5
19	49·2	48	1·2
20	51	49	2
21	54	52·5	1·5
22	53	52	1
23	48	46·3	1·7
24	47·5	45·5	2
25	40·6	39·5	1·1
26	50	48	2
27	50	48·2	1·8
28	53·8	52	1·8
29	52·5	52	0·5
30	34·3	33	1·3
31	29·5	29·3	0·2
32	38	36	2
33	25·3	25	0·3
35	50·2	48·9	1·3
Average Difference			1·36

Tables 3-10 show the centrifuge readings and in Table 15 a statistical analysis of these is given.

TABLE 15.

Statistical Analysis of Percentange Volume Readings.

Bird Number.*	Sex.	Number of Readings.	Minimum Reading.	Maximum Reading.	Average Reading.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1................	Male......................	15	45·8	54·0	50·0	0·7	2·7	5·4
2................	Male......................	15	43·8	50·0	46·3	0·54	2·0	4·3
3................	Male......................	12	46·5	52·0	49·1	0·54	1·9	3·8
4................	Female....................	15	45·4	51·0	49·1	0·4	1·6	3·2
5................	Female....................	13	40·6	54·0	50·4	1·11	4·0	7·9
1, 2 and 3.........	Males......................	42	43·8	54·0	48·4	0·9	2·8	5·8
4 and 5...........	Females....................	28	40·6	51·0	49·7	0·56	3·0	6·2
1 to 5............	Males and Females.........	70	40·6	54·0	48·9	0·34	2·9	5·9
6................	Female....................	14	43·0	52·8	49·0	0·66	2·5	5·1
7 to 11..........	Males......................	5	38·4	47·4	42·0	1·50	3·3	7·8
12 to 17..........	Females....................	6	41·4	49·4	43·0	1·29	3·1	7·2
7 to 17..........	Males and Females.........	11	38·4	49·4	42·6	0·93	3·1	7·2
18 to 22..........	Males and Females.........	6	25·3	41·8	33·0	2·54	6·1	18·5

* For particulars of birds, see pages 428-429.

The average percentage volume reading for the normal birds (1-5) was 48·9±0·3 ranging from 40·6 to 54·0 (standard deviation 2·9; coefficient of variability 5·9 per cent.).

It will be seen that the coefficient of variability ranged from 3·2 to 7·9 and that the exceptionally low percentage volume reading shown by bird No. 5 on 21/1/36 is correlated with the very low erythrocyte count shown by this bird on that date.

The results shown by No. 6 closely approximate those from the normal birds, but the readings obtained with the blood of most of the clinically healthy ostriches (7 to 17) are, as in the case of the worm-infested birds (18 to22), comparatively low.

Perusal of the literature did not reveal percentage volume results for birds. The writer, however, has made a number of percentage volume determinations also on fowl blood and these are appreciably lower than the results from ostrich blood.

Relation between Percentage Volume of Red Cells and Their Number.

Like Neser (1923), the writer determined the ratio of the volume of red cells to their number and in calculating the ratio the count was considered to the nearest 10,000.

The results are listed in Tables 3-10 and a statistical analysis is shown in Table 16. With a few exceptions, the ratios shown by the normal birds (1-5) closely approximate one another, averaging 25·75±0·12 (standard deviation 1·02; coefficient of variability 3·9 per cent.).

Neser (1923), in discussing osmotic pressure of the blood, remarks: " In view of the above statements it is thought unlikely that the osmotic pressure of horse blood can vary much between individuals or in the same individual from time to time. If this is accepted then it must equally be accepted that the volume of the red cells cannot be influenced to any marked extent owing to changes in the osmotic pressure of the blood."

The ostrich does not usually drink much and as the skin of birds does not contain sweat glands (Bradley 1915 and Kaupp 1929) it may be presumed that in the ostrich there is even less change in osmotic pressure of the blood than there is in the blood of an animal like the horse which has a large fluid intake and perspires freely.

As the centrifuge reading can be relied upon to reflect accurately the true volume under the current circumstances the extreme variations are, no doubt, largely due to errors in the count.

Take, for example, the minimum ratio 23·23—obtained with the results shown by bird No. 2 on 26/9/36—and the maximum ratio 28·91—obtained with the results shown by bird No. 5 on 24/11/'36. The respective counts are 1,983,000 and 1,853,000. In discussing the erythrocyte counts, it is shown that they are subject to an error of 10 per cent. and if it is presumed that the above counts were respectively too high and too low by 10 per cent. then the corrected

TABLE 16.

Statistical Analysis of Ratios between Centrifuge Readings and the Red Corpuscular Counts.

Bird Number.*	Sex.	Number of Determinations.	Minimum Ratio.	Maximum Ratio.	Average Ratio.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	23·60	26·94	25·33	0·28	1·09	4·3
2	Male	15	23·23	27·13	25·91	0·22	0·88	3·39
3	Male	12	24·27	27·08	25·52	0·22	0·78	3·05
4	Female	15	24·56	27·47	26·13	0·21	0·83	3·17
5	Female	13	23·34	28·91	25·81	0·38	1·39	5·38
1, 2 and 3	Males	42	23·23	24·27	25·60	0·27	0·94	3·67
4 and 5	Females	28	23·34	28·91	25·98	0·21	1·12	4·31
1 to 5	Males and Females	70	23·23	28·91	25·75	0·12	1·02	3·96
6	Female	14	22·92	26·23	25·11	0·08	0·30	1·19
7 to 11	Males	5	25·56	27·61	26·87	0·38	0·85	3·16
12 to 17	Females	6	25·24	29·65	27·04	0·61	1·51	5·58
7 to 17	Males and Females	11	25·24	29·65	26·96	0·35	1·18	4·37
18 to 22	Males and Females	6	22·18	31·23	25·27	1·28	3·08	12·18

* For particulars of birds, see pages 428–429.

figures would be about 1,785,000 and 2,038,000. The ratios now become 25·84 and 26·35, thus differing only slightly from the average ratio of 25·75. It is, therefore, concluded that also in normal ostrich blood the red cells do not vary appreciably in volume, and that the number of red cells can be as accurately obtained by dividing the percentage volume reading by the figure 25·75 as by counting, provided the samples are centrifuged at the speed at which the centrifuge was run by the writer, and the centrifuge tubes have the same diameter.

The ratios obtained with the results from birds 7-17 are too few to permit of any conclusions being drawn, but the indications are that the red cells of some of them were slightly larger than those of birds 1-5.

The results from the worm-infested birds (18-22) show a comparatively high coefficient of variability.

Minimum and Maximum Resistance of the Red Cells.

The points of minimum and maximum resistance of the red cells of ostrich blood were determined in the following way: Half a c.c. of blood was added to each of a series of test tubes, each containing 10 c.c. sodium chloride solution, but each solution being 0·01 per cent. stronger than the last. Fresh solutions were always made up and the salt was oven-dried before use. After the addition of the blood, the tubes were gently shaken and then allowed to stand for 10 minutes. They were then centrifuged for 10 minutes at a speed of 2,800 revolutions per minute. Within the period specified complete haemolysis and the settling of unhaemolysed erythrocytes and haemoglobin-free stromata took place. It made little or no difference whether the tubes stood for 10 minutes or for 2 hours before being centrifuged and the readings were either the same or differed very slightly, whether the samples were centrifuged or allowed to stand until spontaneous settling of the cells had occurred. It appears that haemolysis is completed within a few minutes, but 10 minutes were allowed to make quite sure. These observations are in agreement with those made on sheep blood by Rossouw (1930).

Kolmer and Boerner (1931) state: " Normally human erythrocytes, carefully collected against injury, can remain for two hours at room temperature in solutions containing 0·42 to 0·44 per cent. sodium chloride before haemolysis begins, whilst under these conditions hemolysis is complete in 0·36 to 0·32 per cent. solutions ". From this statement by Kolmer and Boerner we must conclude that the passage of water into the cells occurs much sooner in the red cells of the ostrich and the sheep than in those of man.

If, according to the method of Rossouw (1930), 1 c.c. ostrich blood be added to 20 c.c. distilled water—and the solution is considered as representing 100 per cent. haemolysis—then a one per cent. haemolysis can usually just be observed with the naked eye, for the solution is only very slightly tinged. When the first evidence of haemolysis was observed, lysis of at least one per cent. of the red cells had therefore already occurred, for the samples were not examined spectroscopically, and the points of minimal resistance recorded indicate haemolysis of at least one per cent. of the cells. Tinging due to the plasma itself can be ignored, for, even when 1 c.c. of plasma is added to 10 c.c. of saline, no colouring of the solution can be detected. Whole blood was used and it was always added to the solutions within half an hour of bleeding. When haemolysis tests were conducted the temperature ranged from 15° C. to 20° C.

Workers in determining the fragility of the red cells used different concentrations of blood in saline. Isaacs (1929), for instance, added 1 drop (a variable quantity) of blood to 2 c.c. saline whereas Rossouw (1930) added 1 c.c. blood to 20 c.c. saline (approximately 1 drop to 1 c.c. saline) and Pepper and Farley (1933) added 1 drop blood to 4 c.c. saline. It is unfortunate that there should be no standard method of performing the test, as accurate comparison of results is often impossible. For example, from the graph given by Rossouw (1930) it will be observed that when 1 c.c. sheep blood was added to 20 c.c. of a 0·72 per cent. sodium chloride solution 3 per cent. haemolysis resulted, and in a 0·67 per cent. sodium chloride solution approximately 12 per cent. haemolysis occurred. The same author states that a 2 per cent. haemolysis gives almost a clear soloution, and a 3 per cent. haemolysis was found to be the lowest practical margin to work with. As the first evidence of haemolysis was observed in the 0·72 per cent. sodium chloride solution, 0·72 might have been considered the point of minimum resistance.

If 0·25 c.c. blood had been added to 20 c.c. of a 0·67 per cent. sodium chloride solution, as had been done by Pepper and Farley (1933), also 12 per cent. of the cells in the 0·25 c.c. blood would have haemolysed, but the solution would have shown a tint similar to that of a 3 per cent. haemolysis in the aforementioned concentration, viz., a colour which can just be observed, and in the latter concentration of blood and saline the point of minimum resistance would have been determined as 0·67.

Wiseman and Bierbaum (1932) have devised a fragility test in which the patient's cells are tested in his own plasma. Centrifugation of the blood is required, but it is not necessary to make up fresh saline each time the test is carried out, or to set up a control test with a known normal blood.

From the results given in Table 17 it will be noted that the point of minimum resistance of the red cells of the ostrich varies from 0·44 to 0·52 with an average of 0·47, and that the point of maximum resistance ranged from 0·25 to 0·3, with an average of 0·26.

Wirth (1931), citing Friedl (1931), gives the points of minimum and maximum resistance of fowl blood as 0·59 and 0·37 respectively, but according to Kleineberger and Carl (1927) the point of minimum resistance of fowl blood varies from 0·4 to 0·47.

TABLE 17.

Resistance of Erythrocytes to Haemolysis. Nos. 1 to 5—Ostriches clinically healthy and found free from disease on Post-mortem Examination. No. 6—Ostrich with club-foot. See pages 428-429.

Bird Number.*	Sex.	Date.	Age.		Minimum Resistance.	Maximum Resistance.
			Months.	Days.		
1	Male	1/10/35	11	1	0·47	0·3
		8/10/35	11	8	0·45	0·29
		30/11/35	13	—	0·44	—
2	Male	26/ 9/35	10	26	0·5	—
		4/10/35	11	4	0·48	0·27
		5/10/35	11	5	0·44	0·26
		19/10/35	11	19	0·5	0·27
		26/10/35	11	26	0·5	—
		11/11/35	12	11	0·5	—
		30/11/35	13	—	0·48	—
3	Male	26/ 9/35	10	26	0·45	0·27
		20/11/35	12	20	0·47	0·27
		7/12/35	13	7	0·46	—
4	Female	24/ 9/35	10	24	0·5	0·3
		1/10/35	11	1	0·47	0·3
		5/10/35	11	5	0·49	0·28
		11/11/35	12	11	0·5	—
		29/11/35	12	29	0·47	—
5	Female	25/ 9/35	10	25	0·49	0·3
		28/ 9/35	10	28	0·52	0·25
		20/11/35	12	20	0·44	0·27
Average 1, 2 and 3	Males	—	—	—	0·47	0·27
Average 4 and 5	Females	—	—	—	0·48	0·28
Average 1 to 5	Males and Females	—	—	—	0·47	0·28
6	Female	28/ 9/35	10	28	0·45	0·27
		20/11/35	12	20	0·44	0·27
		29/11/35	12	29	0·45	—

OSMOTIC PRESSURE ON THE RED CELLS.

The effects of osmotic pressure on the red cells were determined by the method used by Neser (1923), and all the observations were made on whole blood. Repeated observations were made on the blood of six ostriches, and it was found that the cells in the 0·9 per cent. sodium chloride solution always gave either the same reading as those in the plasma, or the reading nearest to those in the plasma; 0·9 per cent. sodium chloride solution can, therefore, for practical purposes be accepted as isotonic for ostrich blood.

As already stated the writer's original intention had been to use isotonic lithium citrate solution as anticoagulant, and it was similarly determined that a 2·8 per cent. solution of this salt is also isotonic for ostrich blood.

Two sets of readings, which also reflect the osmotic effects of certain other solutions on the red cells, are given below:—

(1)

1 c.c. Concentrated blood plus	*Centrifuge readings.*
1 c.c. Plasma	36
1 c.c. 0·7 per cent. sodium chloride solution	39·5
1 c.c. 0·8 ,, ,, ,, ,,	38
1 c.c. 0·9 ,, ,, ,, ,,	36
1 c.c. 1 ,, ,, ,, ,,	35
1 c.c. 1·1 ,, ,, ,, ,,	34·5
1 c.c. 1·2 ,, ,, ,, ,,	34
1 c.c. Ringer's solution	38·5

(2)

1 c.c. Concentrated blood plus	*Centrifuge readings.*
1 c.c. Plasma	33·7
1 c.c. 0·8 per cent. sodium chloride solution	35
1 c.c. 0·9 ,, ,, ,, ,,	34
1 c.c. 1 ,, ,, ,, ,,	33
1 c.c. Ringer's solution	33
1 c.c. Locke's solution	33·5

In order to ascertain if the cells were similarly packed in 0·9 per cent. saline as in plasma, samples of whole blood were centrifuged for one and a half hours at 2,800 r.p.m. The plasma was then pipetted off till near the level of the red cell column and an amount of 0·9 per cent. saline, equal to the amount of plasma drawn off, was added. The tubes were shaken until the saline and the cells were well mixed and then the tubes were recentrifuged at the same speed for the same length of time.

The set of readings given below, which are typical of many other readings, show that the results obtained with whole blood were either the same or differed only slightly from those given by the saline mixtures, and that, in determining the isotonicity of the cells, the percentage volume readings obtained with the cells in the saline are therefore strictly comparable with those given by the cells in the plasma.

Blood.	*Centrifuge Readings.*
Whole blood	51
Cells plus 0·9 per cent. saline	52
Whole blood	48
Cells plus 0·9 per cent. saline	48
Whole blood	36
Cells plus 0·9 per cent. saline	37
Whole blood	50
Cells plus 0·9 per cent. saline	49·5

HAEMOGLOBIN CONTENT.

The haemoglobin content was determined by means of a Newcomer Haemoglobinometer (Bausch and Lomb). The improved model is fitted with a complementary blue filter with the use of which a satisfactory colour match is obtained.

Schultze and Elvehjem (1934) state: "The Newcomer method was never as satisfactory for chicken blood as for mammalian blood because the turbidity of the acid solution makes it difficult to compare the unknown solution with the standard."

These authors describe a method in which the haemoglobin content is also determined colorimetrically and a Newcomer disc is used, but the latter requires restandardisation.

The haemoglobin content of the blood of the normal ostriches (1–5) varied from 14·6 to 17·2 grams per 100 c.c. blood with an average of 16 grams per 100 c.c. blood (Table 18).

Bird No. 22 was received from Bredasdorp District. It showed a heavy worm infestation and it will be noted that the haemoglobin determinations on its blood are appreciably lower than those on the blood of the normal birds.

Cook and Dearstyne (1934) appear to be the only investigators who have previously used the recent modification of the Newcomer Haemoglobinometer. They made haemoglobin determinations on the blood of 78 normal fowls and found the figure to vary from 7 to 17 grams with an average of 10·8 grams per 100 c.c. blood.

LEUCOCYTE COUNTS.

The enumeration of the white cells in ostrich blood is attended with the same difficulties which are encountered in obtaining an accurate count of these cells in the blood of fowls and other birds.

The red cells are nucleated, and when the method ordinarily applied to mammalian blood, of using a diluting fluid containing acetic acid to dissolve the red cells, is used, then, upon the loss of the haemoglobin, the stromata of these cells contract about the nuclei, making it impossible to discriminate between these and the leucocytes.

TABLE 18.

Haemoglobin Content.

Nos. 1 to 5.—Ostriches clinically healthy and found free from disease on post-mortem examination.

No. 6.—Ostrich with club-foot. (See pages 428-429.)

No. 11.—Unthrifty ostrich chick which on post-mortem examination showed marked verminosis.

Bird Number.	Sex.	Date.	Age.		Gm. per 100 c.c. Blood.
			Months.	Days.	
1	Male	4/ 2/37	27	4	16
		20/ 2/37	27	20	17·2
		19/ 5/37	30	19	17·2
2	Male	4/ 2/37	27	4	15·0
		20/ 2/37	27	20	15·3
		19/ 5/37	30	19	15·5
3	Male	4/ 2/37	27	4	15·0
		20/ 2/37	27	20	14·6
		19/ 5/37	30	19	15·2
4	Female	4/ 2/37	27	4	16·0
		20/ 2/37	27	20	16·8
		23/ 3/37	28	23	17·0
		17/ 5/37	30	17	16·0
5	Female	4/ 2/37	27	4	17·0
		20/ 2/37	27	20	16·2
		15/ 5/37	30	15	17·0
Average 1, 2 and 3	Males	—	—	—	15·6
Average 4 and 5	Females	—	—	—	16·5
Average 1 to 5	Males and Females	—	—	—	16·0
6	Female	4/ 2/37	27	4	14·5
		20/ 2/37	27	20	14·4
		17/ 5/37	30	17	14·5
Average	—	—	—	—	14·4
11	Male	5/ 2/37	4	—	11·0
		8/ 2/37	4	3	11·5
		11/ 2/37	4	6	11·0
		19/ 2/37	4	14	11·8
		23/ 2/37	4	18	11·8

On the other hand, if a fluid like Toisson's solution is used, which preserves the red cells, the white cells and the thrombocytes, then again it is impossible to distinguish with certainty between the thrombocytes and the small lymphocytes under the powers of magnification that can be used in conjunction with the counting chamber.

Hayden (1927) used Toisson's solution, but mentions that the method was not satisfactory. Furth (1931), however, remarks that in his experience lymphocytes and thrombocytes can be easily differentiated in Toisson's solution. The solution has the following composition: Distilled water 160 c.c., glycerine (neutral), 30 c.c. sodium sulphate 8 gms., sodium chloride 1 gm., methyl violet 5B. 0·25 gm.

According to Forkner (1929), the thrombocytes of fowl blood may also be confused with red cells in which there is little haemoglobin, but such a difficulty is less likely to occur in ostrich blood, since the disparity in size between the two types is much greater than in the case of the fowl. Various methods have from time to time been advocated to overcome these difficulties, and these are considered below.

According to Shaw (1930),(¹) Warthin (1907) and Schmeisser (1915) resorted to an indirect method. The erythrocytes were counted in the ordinary way, and then in films the ratio between them and the leucocytes was determined. Kleineberger and Carl (1912) counted the erythrocytes, leucocytes and thrombocytes in the counting chamber, and then calculated their respective values from the ratios of these cells found in stained smears.

The indirect methods are obviously subject to great error for they presuppose an absolutely even distribution of the cellular elements; this rarely obtains, as Forkner (1929) and others have pointed out.

Blain (1928) was the first to introduce a direct method for the purpose of staining the leucocytes differentially so that they could be counted in the counting chamber. He utilised the vital staining properties of neutral red and prepared two solutions:—Solution 1 contained neutral red (1 : 5,000), made up in Locke's solution and solution 2 contained 12 per cent. formalin, also made up in Locke's solution. Both solutions were adjusted to a pH of 7·4, and kept at a temperature of 39° C. while in use. The blood was first mixed with the neutral red solution, and the other solution was then added—the white cells take up the neutral red, whereas the red cells do not.

According to Shaw (1930), Blain's method when applied to human blood gives results 20 to 30 per cent. below those obtained by the acetic method. Ohlson (1935) also found lower leucocyte counts with this method than with the others which he tried, and it seems that it is chiefly the lymphocytes which are overlooked by this

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(¹) The writer consulted the publications cited by Shaw. Warthin does not definitely state that he used an indirect method, for he merely remarks: "Toissons solution was found to be the most satisfactory for the blood counting." Schmeisser states that he counted the leucocytes by the indirect method, but he gives no particulars about the method nor any reference.

method. It would also appear that Blain included the thrombocytes either in the red cell or the white cell count, since he does not mention these and, in a further communication (1928) already referred to, he states that he identified no structures in avian blood corresponding to the blood platelets of mammals.

Coates (1928) recommends the solutions which Wright and Kinnicut (1911) used for counting platelets in human blood. Two solutions are employed: Solution 1 contains 1 gm. brilliant cresyl blue in 300 c.c. distilled water, and solution 2 contains 1 gm. potassium cyanide in 1,400 c.c. distilled water. Equal parts of the two solutions are mixed and filtered immediately before use and the resulting solution constitutes the diluting fluid. With subdued illumination, the leucocytes are said to refract the light in such a manner as to render direct counting possible.

Ohlson (1935) found the method unsatisfactory, as the distinction was not well marked between the thrombocytes and the small lymphocytes.

Kyes (1929) recommended a 2 per cent. solution of osmic acid to eliminate the possibility of red cell haemolysis and consequent liberation of nuclei.

Ohlson (1935) states that there is a danger, in this method, of injury to the cornea of the observer, and that no particular advantages were apparent in the trials made.

Forkner (1929) used a diluting fluid, containing 25 mg. neutral red in 100 c.c. of 0·9 per cent. sodium chloride solution, and states that the polymorphonuclear granulocytic cells and the monocytes can easily be differentiated from the other cells; the red cells with little or no haemoglobin, the thrombocytes and the lymphocytes remaining almost entirely unstained. The proportion of granulocytes and monocytes is then determined from a blood smear, and the total leucocyte count is calculated.

Wiseman (1930) states that with Forkner's method the difference in staining intensity of the monocytes frequently occasions inaccuracies through the missing of individual cells, and that diluting fluids, which do not contain a fixative, often fail to preserve the erythrocytes intact. Cook and Dearstyne (1934) used this method, but made no comment on its efficacy.

Kozma (1929) also counted all three types of cells in the counting chamber, using the following solution: Sodium citrate 0·2 gm., ammonium oxalate 0·1 gm., distilled water 100. To this solution is added 8 c.c. of a one per cent. solution of eosin and 4 c.c. of a one per cent. trypanblue solution, and staining of the blood is allowed for 30 minutes before counting is commenced.

In Shaw's (1930) method, 2 solutions are used. Number 1 contains 25 mg. neutral red (Grubler), 0·9 gm. sodium chloride and 100 c.c. distilled water. Number 2 solution contains 12 mg. crystal violet, 3·8 gm. sodium citrate, 0·4 c.c. formaldehyde and 100 c.c. distilled water. Both solutions are filtered and heated to 107° F., the pipette is half filled with No. 1 solution and then filled with solution No. 2. Shaw's observations were made on Homer pigeons, and all the cellular elements were differentiated by their staining reactions in the diluting fluid.

Palmer and Biely (1935) consider Shaw's method satisfactory for the enumeration of the leucocytes in fowl blood, but Ohlson (1935) is of opinion that it is no easier to distinguish the thrombocytes by this method than in Blain's or Toisson's solution. However, he does not state to what extent he considers the discrimination of the thrombocytes possible by Blain's method or in Toisson's solution, but Shaw himself thinks that the thrombocytes cannot be distinguished with certainty from lymphocytes in Toisson's solution and according to Forkner (1929) the thrombocytes may be confused with the lymphocytes in Blain's method.

Wiseman (1930) recommends the use of a solution having the following formula: 95 c.c. Ringer's solution, 5 c.c. formalin and 50 mg. phloxine. This solution stains the granulocytes a bright red in contrast with the other cells which stain much less brilliantly.

The total leucocyte count is then obtained by calculation of the percentage of these cells in the differential count. Wiseman states that maximum staining is obtained within one hour, but suggests that where time is not a factor of importance less phloxine should be used as more dilute solutions (3 mg. dye in 100 c.c. solution) stain the granulocytes almost specifically and the contrast is then at its best, but then a time interval of at least 3 hours is required.

Magath and Higgins (1934) consider that in none of the direct methods are the leucocytes stained in such a manner as to be always distinguishable from the erythrocytes.

Emmel (1935) counted the white cells in a 0·85 per cent. sodium chloride solution tinted with gentian violet, but he says: "There is often difficulty in differentiating the lymphoid precursors of erythrocytes, young erythrocytes and occasionally basophilic erythroblasts when counting erythrocytes and leucocytes in the same chamber."

From this review of the methods advocated for avian leucocyte counting, it will be evident that, as there is no unanimity of opinion about them, the writer had to select one most suitable according to his own observations. After trying them all, he reached the following conclusion: By none of the direct methods could the agranulocytes in ostrich blood be satisfactorily distinguished in the counting chamber from basophil erythroblasts, free red cell nuclei or from the thrombocytes.

The methods of Blain and Shaw are also impracticable when counts have to be made from adult ostriches which cannot be brought into or very near a laboratory, for the diluting fluids have to be at body temperature when mixed with the blood and when counts are being made. When the blood has to be collected eight miles away from the laboratory, as had to be done in this case—for there were no facilities for keeping the ostriches nearer—it is very difficult to conform to such conditions.

The writer's observations on the counting of the leucocytes of ostrich blood in Forkner's solution coincide with Wiseman's on fowl blood.

Wiseman's solution is preferred for leucocyte counting in ostrich blood for the following reasons: The granules in the granulocytes stain in such a distinctive manner, that these cells can be readily identified. This counting fluid is particularly suitable for counting white cells in ostrich blood, since ostrich blood contains a high percentage of granulocytes. The cells are stained satisfactorily within a short time and they are preserved for a long time. Counts made weeks later agreed, within limits of counting errors, with those made shortly after mixing. The counts can, therefore, be made at leisure at any convenient time. Since the staining qualities of the solution do not deteriorate, it can be prepared in large quantity if the bottle is kept well corked—to prevent evaporation of the formalin—and shaken every time before use.

Ohlson (1935), who made a comparative study (on fowl blood) of various methods in order to gauge their accuracy for both erythrocyte and leucocyte counts, found that Blain's method gave the most consistent results for leucocytes, Wiseman's method being placed third. He favours Wiseman's solution when dealing with pathological blood, stating that all the direct methods failed with the blood of an erythroleucotic chicken—the immature erythrocytes being confused with leucocytes—whereas in Wiseman's solution all the haemoglobin—bearing cells are stained in a characteristic manner; also that Wiseman's technique gave the lowest coefficient of variation in the erythrocyte counts and that it showed a tendency to give higher red cell counts. He also remarks: "An explanation for the tendency of the Wiseman method to give higher erythrocyte and leucocyte values is lacking. The erythrocytes are fixed and more distinctly stained than with the other methods." (The writer used Wiseman's solution as diluent for erythroleucotic fowl blood but also in this solution haemocytoblasts and basophil erythroblasts could not be differentiated from non-granular leucocytes.)

Wiseman's solution was used in making both the erythrocyte and the leucocyte counts, but the solution was slightly modified by substituting 0·9 per cent. sodium chloride solution containing 20 gm. sodium bicarbonate per 100 c.c. for Ringer's solution. The modified Wiseman diluting fluid, therefore, has the following formula: Phloxine 50 mg., sodium bicarbonate 20 mg., sodium chloride 0·9 mg., formalin 5 c.c., distilled water 95 c.c.

This solution proved as satisfactory as that containing Ringer's solution and its preparation entailed less work. (Locke's solution is isotonic for ostrich blood and in Wiseman's solution it is also a suitable substitute for Ringer's solution). Staining was allowed for at least one hour before the cells were counted. It is possible to recognize unstained granulocytes, but counting is greatly facilitated when the cells are well stained.

Turbidity may appear in an old sample of diluent, but this can be easily removed by adding a small quantity of sodium bicarbonate.

A difficulty with all the methods is that solutions sufficiently concentrated to preclude a large counting error cannot be used. With all the methods the erythrocytes are preserved and when the red cell count is somewhat high it is necessary to use dilutions of 1 in 200.

However, in normal ostrich blood—though the red cells are comparatively large—counts can be made wih ease in dilutions of 1 in 100. Dilutions of 1 in 50 are also suitable but it was customary also to count the red cells in the same chamber and in this concentration accurate counting is difficult and, if prolonged, a severe strain on the observer's eyes as the cells are then close together. But as the numerical error resultant on multiplication is halved by using the 1 in 50 dilution it may be used with advantage when the intention is to count the granulocytes only for the erythrocytes are not so close together as to obscure the granulocytes.

It was customary always to have ready for use a number of bottles each having a capacity of a little over 100 c.c. and containing 99 c.c. of the diluent, for the dilutions were effected by using 1 c.c. blood to 99 diluent. Only pipettes and burettes were used for which certificates of accuracy had been issued by the United States Bureau of Standards.

Another drawback, which the writer has not seen mentioned by previous workers, is the marked tendency which the leucocytes—both in ostrich blood and also in fowl blood—show to clump after the blood has been drawn. The intention at first was to collect the ostrich blood in an anticoagulant and to make the subsequent dilutions when convenient, as Neser (1923) did. But it was found that, unless the blood is mixed with the diluting fluid immediately after it has been drawn, clumping of the white cells and particularly of the heterophiles is so marked as to vitiate the count; as many as 20 or more leucocytes may be together, usually intermixed with thrombocytes. When the mixing takes place immediately after the blood has been drawn, clumping is usually absent or only slight.

The best results were obtained when a red cell pipette was used, for then the interval between the drawing of the blood and the mixing was reduced to a minimum, but using a pipette on the intractable ostrich is usually so difficult that it may be considered impracticable.

It was ascertained that clumping will take place also in fowl blood if there is some delay in filling the pipette, or if the blood is first received into an anti-coagulant. Many diluting fluids besides Wiseman's and Wiseman's (modified) were tried, namely, Hayem's, Toisson's, Tyrode's, Locke's, Ringer's, 0·9 per cent. sodium chloride and also the bird's own plasma, but clumping was no less marked in any of them.

The bottle containing the blood and the diluting fluid was usually shaken for about three minutes before the fluid was introduced into the chamber, but even shaking for longer periods had no effect in reducing the clumping; on the contrary it often increased it. The fluid was also shaken with beads, but this also proved ineffective. Blood, allowed to stand for half an hour, was added to a

1 per cent. solution of acetic acid, but clumping was marked. Human, equine and bovine blood, similarly treated, did not show agglutination, so that this quality seems to be characteristic only of avian blood.

All the anticoagulants mentioned under "Preventing Coagulation of the Blood" were tried but without success in overcoming this disadvantage. The mixing had, therefore, to be always made at the place of bleeding. When only the red cells have to be counted the blood may be diluted hours later if kept cool in the interim.

Forkner (1929) mentions the rapidity with which coagulation takes place in the blood of the fowl as one of the difficulties in securing an accurate count of the white cells. The writer experienced no difficulty in filling the pipettes when making counts of fowl blood, as the blood never coagulated before it could be properly diluted, but it was observed that, when the dilution of fowl blood did not take place immediately, clumping was at times also marked. It may have been this that was responsible for the difficulty which Forkner experienced, rather than that fowl blood coagulates too soon.

At first a Burker counting chamber was used, but later it was replaced by a Levy-Hausser counting chamber, guaranteed to be within the tolerance for accuracy established by the United States Bureau of Standards. This counter, which is recommended by Kolmer and Boerner (1931), has features which make counting much easier and which are not found with some other counters.

The total number of granulocytes in at least four of the entire ruled areas, viz., in 3·6 c.mm. of the mixture, was always counted and so were all the red cells in at least 0·12 c.mm. Errors due to smallness of samples were thus reduced to a minimum. The counting of the red cells was spread over four of the entire ruled areas, the two rectangular areas, each measuring 3 mm. x 0·05 mm. and crossing each other in the centre of the ruled area, being used, i.e., the cells in 0·03 c.mm. of the mixture were counted in each chamber. In this way a better average count check was ensured.

The difference between 2 sets of red cell counts thus made seldom varied by more than 5 per cent. but differences up to 10 per cent. were noted in spite of the utmost care in making the preparations as recommended by Neser (1923) and others to ensure accurate counting. Marked differences were sometimes obtained when smaller samples were counted or when the count was spread over two ruled areas only.

All the diluting fluids mentioned on page 479 were tried to see whether more regular distribution of the red cells could be obtained but none of them proved to be better than the diluent used.

Neser's method of using an ordinary pipette drawn from glass tubing for filling the counting chamber was adopted for ostrich blood. The point should be cut in such a way that the fluid flows readily in between the two surfaces but without flooding the chamber when the forefinger is slightly withdrawn from the upper end of the pipette.

It was found most convenient to use a 6 x ocular and an 8 mm. lens and a strong white light for illumination.

TABLE 19.

Statistical Analysis of Leucocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Count per c.mm.	Maximum Count per c.mm.	Mean Count per c.mm.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1................	Male....................	15	12.326	29.255	21,985	846	3,279	14·9
2................	Male....................	15	13.157	24.751	19.859	829	3,210	16·1
3................	Male....................	12	19.436	31.303	22.708	655	2,267	9·9
4................	Female..................	15	14,157	32.273	20.733	974	3.773	18·1
5................	Female..................	13	15.880	27,495	20,179	798	2.875	14·2
1, 2 and 3.........	Males.....................	42	12,326	31.303	21.432	489	3,172	14·8
4 and 5...........	Females..................	28	14,157	32.273	20.476	736	3,892	19·0
1 to 5............	Males and Females.........	70	12.326	32,273	21.050	419	3,482	16·5
6................	Female....................	14	17.422	45.372	29.562	3,441	9,130	30·8
7 to 11..........	Males.....................	5	8,409	16,146	13,125	1,439	3,166	24·1
12 to 17..........	Females..................	6	7.868	24,570	14.066	2,449	5.878	41·7
7 to 17..........	Males and Females.........	11	7.868	24.570	13.638	1,264	4,172	30·5
18 to 22..........	Males and Females.........	6	8,697	30.033	17,210	2,803	6,729	39·0

* For particulars *re* birds, see pages 428–429.

The leucocyte cells counts are given in Tables 3–10 and a statistical analysis of the data is shown in Table 19. The results show that the mean leucocyte count of all the results from the normal birds (1–5) was 21,050 ±419 with a range of 12,326 to 32,273 (standard deviation 3,482; coefficient of variability 16·5 per cent.).

It will be seen that the coefficient of variability is much higher than that for the erythrocytes. Palmer and Biely (1935), who made leucocyte counts of 100 normal S.C. white Leghorn hens, reported a coefficient of variability of 20·77, while, according to these writers, the leucocyte counts made by Cook and Dearstyne (1934) on 75 normal Rhode Island Red and Barred Plymouth Rock fowls show a coefficient of variability of 55·09.

The average leucocyte count for bird No. 6 is comparatively high and so is the coefficient of variability of the results from this bird. It would, therefore, appear that the leucocyte count was affected by the defect from which the bird suffered.

The clinically healthy birds (7–17) showed a mean leucocyte count which is significantly lower than that of the normal birds and the coefficient of variability of their counts is comparatively high. The results from the worm-infested birds (18–22) fluctuate considerably between the figures 8,697 and 30,033.

As in the case of the erythrocyte counts, the leucocyte counts cannot be correlated with the age or the sex of the birds.

Hayem (1879) recorded for the ostrich a leucocyte count of 9,000 per c.mm.. (Table 1).

Differential Counts.

In the differential counts at least 400 cells were always counted in each case and the counts were recorded on sheets as were used by Neser (1923).

Table 20 shows sixteen differential counts. These were obtained from the same smear prepared from ostrich blood by counting 100 leucocytes each time. A statistical analysis of these counts which can be regarded as typical gives the following results for example in respect of the lymphocytes and the heterophiles: By counting 100, 200, 300 and 400 leucocytes the chances are 20:1 that the errors associated with the lymphocyte counts are respectively 8·0, 5·1, 4·6 and 4·0; the errors associated with the heterophile counts are respectively 11·2, 8·0, 6·4 and 5·6. It is evident that by counting 400 leucocytes, fairly accurate results may be expected.

The differential counts were always made vertically across the smear, and an Ehrlich eye-piece was used for limiting the field, thus rendering critical observation of the cells at the edges of the field possible.

The differential counts are tabulated in Tables 3-10 and statistical analyses are shown in Tables 21-25. Examination of the results shows that the lymphocyte counts of the normal birds (1-5) varied from 19·2 to 37·0 per cent., averaging 26·8±0·5 per cent.

(standard deviation 4·1; coefficient of variability 15·3 per cent.). The average lymphocyte count of birds 1-5 is significantly higher than that of birds 7-17.

TABLE 20.

Differential Counts from same Smear prepared from Ostrich Blood.

Lymphocytes.	Monocytes.	Heterophiles.	Eosinophiles.	Basophiles.
%	%	%	%	%
31	5	46	7	11
38	7	41	4	10
32	5	47	3	13
26	1	52	7	14
28	3	52	6	11
26	5	56	6	7
28	1	59	5	7
27	5	55	5	8
20	4	61	4	11
30	4	49	8	9
26	3	56	6	9
22	5	59	11	3
30	6	45	8	11
29	5	48	1	17
32	2	46	5	15
25	5	53	7	10
Av. 28·1	4·1	51·5	5·7	10·3

The monocyte counts of all the results from birds 1-5 varied from 0·5 to 8·5 per cent., averaging 3·0±0·3 per cent. (standard deviation 1·2; coefficient of variability 40·0 per cent.). Though the counts show high variability the averages do not differ much.

The heterophile counts shown by birds 1-5 varied from 40·5 to 78·5 per cent., averaging 59·1±0·9 per cent. (standard deviation 7·7; coefficient of variability 13·0 per cent.). This average differs appreciably from that obtained with the results from bird No. 6 and from the average shown by birds 7 to 17.

The eosinophile counts of birds 1-5 varied from 0 to 19·5 per cent., averaging 6·3±0·5 per cent. (standard deviation 4·0; coefficient of variability 63·5 per cent.). These cells show the highest variability.

The averages for birds 5 and 6 are exceptionally low while the average figure for birds 7-17 is exceptionally high compared with the mean count shown by birds 1-5. As stated on page 429 it is most likely that all the birds Nos. 7-17 were worm-infested and the high eosinophile counts of these birds may possibly be correlated with verminosis; however, some of the worm-infested birds (18–22) showed comparatively low eosinophile counts.

The basophile counts of birds 1-5 varied from 1·0 to 10·5 per cent., averaging 4·7±0·3 per cent. (standard deviation 2·5; coefficient of variability 53·2 per cent.). This average does not differ appreciably from that shown by birds 7-17. The few differential counts on the worm-infested birds (18-22) were most erratic.

TABLE 21.

Statistical Analysis of Lymphocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1................	Male......................	15	21·0	32·5	26·3	1·0	4·0	15·2
2................	Male......................	15	22·0	37·0	28·3	0·9	3·5	12·4
3................	Male......................	12	23·0	36·2	27·6	0·9	3·4	12·3
4................	Female....................	15	19·2	35·5	28·2	0·9	3·7	13·1
5................	Female....................	13	17·0	33·5	23·1	1·2	4·4	19·0
1, 2 and 3.........	Males......................	42	21·0	37·0	27·4	0·5	3·6	13·1
4 and 5...........	Females..................	28	19·2	35·5	25·9	1·0	4·6	17·8
1 to 5............	Males and Females.........	70	19·2	37·0	26·8	0·5	4·1	15·3
6................	Female....................	14	14·2	28·2	21·4	1·4	5·4	25·2
7 to 11...........	Males......................	5	8·7	15·7	12·8	1·2	2·7	21·0
12 to 17..........	Females..................	6	4·0	13·7	8·3	1·6	3·8	45·8
7 to 17..........	Males and Females.........	11	4·0	15·7	10·3	1·2	4·0	38·8
18 to 22..........	Males and Females.........	6	7·2	45·7	26·3	0·6	14·0	53·2

* For particulars of birds, see pages 428-429.

TABLE 22.

Statistical Analysis of Monocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	1·7	8·5	3·5	0·4	1·5	42·8
2	Male	15	1·7	6·0	3·5	0·3	1·3	37·1
3	Male	12	1·2	4·2	2·8	0·2	0·8	28·5
4	Female	15	0·5	5·5	2·5	0·3	1·2	48·0
5	Female	13	1·5	4·2	2·7	0·2	0·9	33·3
1, 2 and 3	Males	42	1·2	8·5	3·3	0·2	1·3	39·3
4 and 5	Females	28	0·5	5·5	2·6	0·2	1·1	42·3
1 to 5	Males and Females	70	0·5	8·5	3·0	0·3	1·2	40·0
6	Female	14	0·7	6·2	3·4	0·4	1·6	47·0
7 to 11	Males	5	1·0	4·5	3·1	0·6	1·3	41·9
12 to 17	Females	6	2·0	4·5	3·2	0·4	1·1	34·3
7 to 17	Males and Females	11	1·0	6·2	3·2	0·3	1·1	34·3
18 to 22	Males and Females	6	2·0	9·5	4·4	1·2	2·8	63·6

* For particulars of birds, see pages 428–429.

Table 23.

Statistical Analysis of Heterophile Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	41·5	64·0	54·2	1·5	6·0	11·1
2	Male	15	46·3	63·2	56·1	1·4	5·6	10·0
3	Male	12	54·2	65·5	60·4	1·1	3·7	6·1
4	Female	15	40·5	71·5	58·0	2·0	7·9	13·6
5	Female	13	53·0	78·5	68·1	1·8	6·6	9·7
1, 2 and 3	Males	42	41·5	64·0	56·6	0·9	5·8	10·2
4 and 5	Females	28	40·5	78·5	62·7	1·7	8·9	14·2
1 to 5	Males and Females	70	40·5	78·5	59·1	0·9	7·7	13·0
6	Female	14	61·0	81·0	71·1	1·8	6·7	9·4
7 to 11	Males	5	62·0	73·0	68·3	1·8	4·0	5·8
12 to 17	Females	6	68·7	83·2	74·4	2·4	5·8	7·8
7 to 17	Males and Females	11	62·0	83·2	71·6	1·7	5·7	8·0
18 to 22	Males and Females	6	47·5	89·7	63·0	6·8	16·4	26·0

* For particulars of birds, see pages 428-429.

TABLE 24.

Statistical Analysis of Eosinophile Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1.................	Male......................	15	2·7	19·7	7·8	1·3	5·0	64·1
2.................	Male......................	15	3·5	14·7	7·6	0·8	3·0	39·4
3.................	Male......................	12	3·0	8·7	6·4	0·5	1·7	26·6
4.................	Female....................	15	2·0	16·5	7·3	1·2	4·6	63·0
5.................	Female....................	13	0	3·7	1·9	0·3	1·2	63·1
1, 2 and 3.........	Males......................	42	2·7	19·7	7·3	0·5	3·5	47·9
4 and 5...........	Females....................	28	0	16·5	4·8	0·8	4·4	91·7
1 to 5............	Males and Females.........	70	0	19·5	6·3	0·5	4·0	63·5
6.................	Female......................	14	0·2	4·5	1·7	0·4	1·6	94·1
7 to 11...........	Males......................	5	6·0	18·0	12·4	2·2	4·8	38·7
12 to 17...........	Females....................	6	3·2	18·7	10·1	2·2	5·2	51·5
7 to 17..........	Males and Females.........	11	3·2	18·7	11·1	1·5	4·9	44·1
18 to 22..........	Males and Females.........	6	0·2	13·7	4·4	1·5	5·3	120·4

* For particulars of birds, see pages 428-429.

TABLE 25.

Statistical Analysis of Basophile Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	3·5	10·5	8·1	0·5	1·8	22·2
2	Male	15	1·7	8·5	4·4	0·5	2·0	45·4
3	Male	12	1·0	4·0	2·7	0·3	1·0	37·0
4	Female	15	1·2	6·5	4·0	0·1	1·7	42·5
5	Female	13	1·0	6·5	4·0	0·5	1·8	45·0
1, 2 and 3	Males	42	1·0	10·5	5·2	0·4	2·8	53·8
4 and 5	Females	28	1·0	6·5	4·0	0·3	1·7	42·5
1 to 5	Males and Females	70	1·0	10·5	4·7	0·3	2·5	53·2
6	Female	14	1·0	4·5	2·3	0·3	1·1	47·8
7 to 11	Males	5	1·2	4·0	3·0	0·5	1·2	40·0
12 to 17	Females	6	0·5	10·0	3·7	1·4	3·4	91·9
7 to 17	Males and Females	11	0·5	10·0	3·4	0·8	2·6	76·5
18 to 22	Males and Females	6	0	3·0	1·5	0·4	1·1	73·3

* For particulars of birds, see pages 428–429.

Thrombocyte Counts.

The thrombocytes were counted by the method employed by Magath and Higgins (1934). The ratio between these and the leucocytes was computed from the number of thrombocytes enumerated each time 200 leucocytes were counted in the smear.

As the thrombocytes are very unevenly distributed the ratio obtained is only approximate, even when 200 leucocytes have been counted. The method is far from satisfactory but there is no better method of obtaining total thrombocyte counts of ostrich blood.

The counts are listed in Tables 3–10 and statistical determinations are given in Table 26. The results from the normal birds (1–5) ranged from 2,478 to 20,086, averaging 10,521 ± 427 (standard deviation 3,545; coefficient of variability 33·6 per cent.). The numbers of thrombocytes and leucocytes stand in a ratio of about 1 to 2.

Kleineberger and Carl (1912) record a thrombocyte count of 22,000 to 130,000 per c.mm. for the fowl while according to Fritsch (1920), cited by Wirth (1931), fowl blood contains very few thrombocytes, and Blain (1928), as already stated, apparently did not see any. Magath and Higgins (1934) reported for the duck an average thrombocyte count of 30,706 ± 703 with a coefficient of variability of 32 per cent. Hayem (1879 and 1889) recorded counts of 11,500 and 11,600 for the ostrich. It will be seen that these figures come very near to the averages obtained by the writer.

VISCOSITY.

Hess's viscosimeter was used. Naegeli (1921) and others give a detailed description of the apparatus. The readings listed in Table (27) are those of citrated blood but tests carried out with whole, citrated, and heparinised blood showed no significant differences. The necessary corrections were made when tests were conducted outside the temperature range of 17° C.–23° C. in which the apparatus gives correct readings.

The viscosity of the blood of the normal ostriches (1–5) varied from 4·0 to 5·0, with an average of 4·5, the corresponding figures for the plasma being 1·5 to 1·8 and 1·6.

The results from the worm-infested birds (18–22) are comparatively low. These birds showed also low erythrocyte counts (Table 10) and on post-mortem examination were found to be severely infested with worm parasites.

Wirth (1931), quoting Kruger (1925), states that the viscosity of fowl serum is 1·4 and that of duck serum 1·24, but figures for avian blood or plasma are not given.

SPECIFIC GRAVITY.

The specific gravity was determined at a temperature of 15° C. with the use of a pycnometer. The blood and plasma were brought to the desired temperature in a thermostat on occasions when the

TABLE 26.

Statistical Analysis of Thrombocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Count per c.mm.	Maximum Count per c.mm.	Mean Count per c.mm.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	6,014	14,076	10,073	725	2,807	27·8
2	Male	15	2,478	19,623	10,456	960	3,718	35·5
3	Male	12	5,562	14,803	10,802	792	2,741	25·3
4	Female	15	4,752	20,086	11,758	1,156	4,476	38·0
5	Female	13	2,686	15,958	9,428	1,103	3,971	42·1
1, 2 and 3	Males	42	2,478	19,623	10,418	476	3,084	29·6
4 and 5	Females	28	2,686	20,086	10,677	793	4,198	39·4
1 to 5	Males and Females	70	2,478	20,086	10,521	427	3,545	33·6
6	Female	14	4,061	26,650	12,439	1,667	6,235	50·1
7 to 11	Males	5	8,946	17,415	13,091	1,375	3,026	23·1
12 to 17	Females	6	7,720	15,336	12,693	1,250	3,002	23·6
7 to 17	Males and Females	11	7,720	17,415	12,890	867	2,864	22·2
18 to 22	Males and Females	6	4,650	15,394	11,135	1,780	4,274	38·3

* For particulars of birds, see pages 428-429.

atmospheric temperature in the room was not exactly 15° C. It will be noted from the determinations given in Table 28 that the specific gravity of the blood of the ostrich varies from 1·060 to 1·065, the average being 1·063. The corresponding figures for the plasma are 1·021 to 1·024 and 1·022.

According to Kruger, cited by Wirth (1931), the specific gravity of the blood of the fowl is 1·0545 and that of its serum 1·0232. The corresponding figures as given by him for the blood and the serum of the goose are 1·0549 and 1·0202—for the duck 1·0563 and 1·0202 and for the guinea-fowl 1·0577 and 1·0214.

Wirth (1931) gives a number of specific gravity determinations—by various workers—of the blood and the serum of a number of animals. The average figure obtained for the blood of the ostrich exceeds the average figures listed by Wirth, the nearest to the average being that given by Augsburger (1919), viz., 1·062 for the horse. Other workers, however, give lower values for the horse.

TABLE 27.

Viscosity Determinations.—Nos. 1-5—*Ostriches clinically healthy and found free from disease on Post-mortem Examination. No.* 6—*Ostrich with club-foot.* (*See pages* 428-429.) *Nos.* 18-22—*Unthrifty Ostrich Chicks which on Post-mortem Examination showed marked Verminosis.*

Bird Number.	Sex.	Date.	Age.		Viscosity Blood.	Viscosity Plasma.
			Months.	Days.		
1	Male	30/11/35	13	—	4·6	—
		12/12/35	13	12	4·8	—
		24/ 3/36	16	24	4·7	1·7
		9/ 5/37	18	9	4·8	1·7
2	Male	7/12/35	13	7	4·1	1·7
		12/12/35	13	12	4·4	1·6
		9/ 5/36	18	9	4·6	1·7
		26/ 8/36	21	26	4·3	—
3	Male	7/12/35	13	7	4·5	—
		9/12/35	13	9	4·2	1·6
		12/12/35	13	12	4·3	1·6
		7/ 9/36	22	7	4·9	1·7
4	Female	29/11/35	12	29	4·1	—
		12/12/35	13	12	4·3	1·6
		24/ 3/36	16	24	4·6	1·5
		9/ 5/36	18	9	4·6	1·6
5	Female	15/11/35	12	15	4·0	1·7
		20/11/35	12	20	4·7	1·8
		12/12/35	13	12	4·7	1·8
		7/ 9/36	22	7	5·0	—

TABLE 27—(*continued*).

Bird Number.	Sex.	Date.	Age.		Viscosity Blood.	Viscosity Plasma.
			Months.	Days.		
Average 1, 2 and 3.......	Males......	—	—	—	4·5	1·6
Average 4 and 5.........	Females...	—	—	—	4·5	1·6
Average 1 to 5...........	—	—	—	—	4·5	1·6
6......................	Females...	20/11/35	12	20	5·5	—
		29/11/35	12	29	4·1	1·7
		7/ 9/36	22	7	5·4	1·8
		1/12/36	25	1	4·8	1·7
18......................	—	15/ 3/36	4	—	3·2	—
19......................	—	19/ 3/36	4	—	2·3	—
20......................	—	20/ 3/36	4	—	2·3	—
21......................	—	22/ 3/36	4	—	2·0	—
22......................	—	8/ 2/37	4	—	3·5	—

TABLE 28.

Specific Gravity Determinations.—Ostriches clinically healthy and found free from Disease on Post-Mortem Examination.

Bird Number and Sex.	Date.	Age.		Temperature at which Specific Gravity Determined.	Specific Gravity Blood.	Specific Gravity Plasma.
		Months.	Days.			
1 Male................	24/ 3/36	16	24	15° C.	1·064	—
	5/ 6/36	19	5	,,	1·065	1·023
2 Male................	9/ 5/36	18	9	15° C.	1·060	1·022
3 Male................	9/ 5/36	18	9	15° C.	1·064	1·023
4 Female..............	24/ 3/36	16	24	15° C.	1·064	—
	31/ 1/36	19	3	,,	1·066	1·021
5 Female..............	9/ 5/36	18	9	15° C.	1·062	1·022
	27/ 5/36	19	—	15° C.	1·062	1·024
Average Males.........	—	—	—	—	1·063	1·022
Average Females.......	—	—	—	—	1·063	1·022
Average 1 to 5 Males and Females	—	—	—	—	1·063	1·022

INORGANIC PHOSPHORUS, CALCIUM, SODIUM, POTASSIUM AND MAGNESIUM CONTENT.

Theiler, Green, du Toit, and others in the series of articles "Studies in Mineral Metabolism" 1927, et. seq., have shown that in many parts of South Africa cattle and sheep suffer from marked aphosphorosis resultant on phosphorus deficiency in the pastures and that phosphorus feeding is an essential factor in successful cattle and sheep farming. It seems, therefore, that phosphorus feeding to ostriches grazing over areas where other animals show aphosphorosis might profitably be investigated. Malan (1930) has shown that phosphorus deficiency, even in the earliest stages may be diagnosed by determining the inorganic phosphorus content of the blood.

As the ostriches Nos. 1 to 5 could be regarded as entirely free from disease and as they always received a liberal supply of bones and other necessary foodstuffs, there was a good opportunity for obtaining data which might contribute toward establishing what levels of phosphorus and of the other mineral elements mentioned above may be considered normal in the blood of the ostrich. Blood analyses were, therefore, made from these birds and—for the purpose of comparison—from bird No. 6, and also from birds (Nos. 7-17) grazing on natural pasture in the Bredasdorp district.

The calcium content was determined particularly with the object of ascertaining whether the prolonged coagulation time could be associated with a low calcium content of the blood, but, as has been shown under "Coagulation of the Blood", this would not appear to be so.

The analyses were carried out under the supervision of Dr. A. I. Malan, Head of the Department of Bio-chemistry, Onderstepoort Laboratories, Pretoria, by his staff. The methods advocated by Malan and van der Lingen (1931) were employed. The writer collected the blood, precipitated the proteins and forwarded the trichloracetic acid filtrate to Onderstepoort.

The results are tabulated in Table 29 and the following are the average values per 100 c.c. blood of all the results obtained from the normal birds (1-5). Inorganic phosphorus 9·1 ± 0·3 mgm., calcium 10·1 ± 0·4 mgm., magnesium 7·6 ± 0·4 mgm., sodium 273·7 ± 13·0 mgm. and potassium 196·6 ± 6·0 mgm.

The inorganic phosphorus content of the blood of all five birds was comparatively low on 29/7/35, and it is not apparent why they showed relatively low values on that date. At no time was any alteration made in their food and it does not seem as if the values can be associated with their age, for bird No. 6 at the age of six months and sixteen days—before it had sustained the injury—showed a higher value than it did at the age of nine months and two days. The results are, however, suggestive of a seasonal variation.

It will be noted that the average values of all the above-named elements obtained from bird No. 6 and those of inorganic phosphorus and calcium shown by the results from the clinically healthy birds (7-17) do not differ appreciably from the mean values of all the results from birds 1–5.

TABLE 29.

Analytical Results.

Nos. 1 to 5.—Ostriches clinically healthy and found free from disease on post-mortem examination. Kept on farm Mariendahl, Stellenbosch District. (See pages 428-429.)

No. 6.—Ostrich with club-foot. Kept on farm Mariendahl, Stellenbosch District. (See pages 428-429.)

Nos. 7 to 13.—Clinically healthy semi-mild ostriches on which post-mortem examinations were not conducted. From farm Negwag, Bredasdorp District. (See page 429.)

	Bird No.	Sex.	Date.	Age.		Mgm. per 100 c.c. Blood.				
						Inorg. P.	Ca.	Mg.	Na.	K.
				Months.	Days.					
	1	Male.............	29/ 7/35	9	2	5·8	10·0	—	—	—
			12/12/35	13	17	10·2	8·2	7·8	250·0	211·5
			17/ 4/36	17	24	8·9	8·5	—	—	—
			20/ 2/37	28	4	10·2	8·1	—	—	—
	2	Male.............	29/ 7/35	9	2	6·7	11·0	—	—	—
			12/12/35	13	17	10·2	10·3	8·4	270·0	177·5
			17/ 4/36	17	24	9·2	9·4	—	—	—
			20/ 2/37	28	4	10·1	8·9	—	—	—
	3	Male.............	29/ 7/35	9	2	7·6	10·7	—	—	—
			12/12/35	13	17	11·4	13·3	7·1	256·2	202·0
			17/ 4/36	17	24	9·3	10·0	—	—	—
			20/ 2/37	28	4	10·4	8·3	—	—	—
	4	Female...........	29/ 7/35	9	2	6·7	10·1	—	—	—
			12/12/35	13	17	10·2	11·6	7·7	270·0	189·0
			17/ 4/36	17	24	9·2	10·3	—	—	—
			20/ 2/37	28	4	10·3	8·6	—	—	—
	5	Female...........	29/ 7/35	9	2	6·9	10·0	—	—	—
			12/12/35	13	17	10·2	14·0	7·4	322·5	203·0
			17/ 4/36	17	24	9·0	14·2	—	—	—
			20/ 2/37	28	4	10·1	8·3	—	—	—
Averages..............	1, 2, 3	Males.............	—	9	2	6·7	10·5	—	—	—
,, ...	,,	,, ...	—	13	17	10·6	10·6	7·7	258·7	197·0
,, ...	,,	,, ...	—	17	24	9·1	9·3	—	—	—
,, ...	,,	,, ...	—	28	4	10·2	8·4	—	—	—
Averages..............	4, 5	Females..........	—	9	2	6·8	10·5	—	—	—
,, ...	,,	,, ...	—	13	17	10·2	12·8	7·5	296·2	196·0
,, ...	,,	,, ...	—	17	24	9·1	12·2	—	—	—
,, ...	,,	,, ...	—	28	4	10·2	8·4	—	—	—

TABLE 29 (*continued*).

	Bird No.	Sex.	Date.	Age.		Mgm. per 100 c.c. Blood.				
						Inorg. P.	Ca.	Mg.	Na.	K.
				Months.	Days.					
Averages...............	1 to 5	Males and Females..	—	9	2	6·7	10·3	—	—	—
"	"	" " ..	—	13	17	10·4	11·4	7·6	273·7	196·6
"	"	" " ..	—	17	24	9·1	10·4	—	—	—
"	"	" " ..	—	28	4	10·2	8·4	—	—	—
Averages, all Results.....	1 to 5	—	—	—	—	9·1	10·1	7·6	273·7	196·6
Standard Error..........	—	—	—	—	—	0·3	0·4	0·4	13·0	6·0
Standard Deviation......	—	—	—	—	—	1·5	1·6	0·9	28·6	13·3
Coefficiency of Variability	—	—	—	—	—	16·4	15·8	11·8	10·4	6·7
	6	Female............	15/ 5/35	6	16	8·2	10·5	8·2	238·0	181·5
			29/ 7/35	9	2	6·9	10·1	—	—	—
			12/12/35	13	17	11·6	13·1	7·7	270·0	212·0
			17/ 4/36	17	24	9·9	10·2	—	—	—
			20/ 2/37	28	4	10·4	8·1	—	—	—
Averages...............	—	—	—	—	—	9·4	12·4	7·9	254·0	196·7
Standard Error..........	—	—	—	—	—	0·6	0·7	—	—	—
Standard Deviation......	—	—	—	—	—	1·5	1·7	—	—	—
Coefficiency of Variability	—	—	—	—	—	15·9	13·7	—	—	—
	7	Male..............	1/ 5/36	Over 3	Years.	10·5	10·2	—	—	—
	8	"	1/ 5/36	"	"	7·3	8·6	—	—	—
	9	"	1/ 5/36	"	"	9·7	9·8	—	—	—
	10	"	1/ 5/36	"	"	8·6	8·7	—	—	—
	11	Female............	1/ 5/36	"	"	8·1	9·3	—	—	—
	12	"	1/ 5/36	"	"	7·9	8·7	—	—	—
	13	"	1/ 5/36	"	"	10·0	9·3	—	—	—
Averages...............	7 to 13	—	—	—	—	8·9	9·2	—	—	—
Standard Error..........	—	—	—	—	—	0·4	0·2	—	—	—
Standard Deviation......	—	—	—	—	—	1·2	0·6	—	—	—
Coefficient of Variability	—	—	—	—	—	13·4	6·0	—	—	—

Malan (1930) recorded for the ostrich a value of 5·5 mgm. inorganic phosphorus per 100 c.c. blood (Table 1) and, according to this writer, fowl blood contains 2 mgm. and pigeon blood 1·6 mgm. inorganic phosphorus per 100 c.c. blood. Under the heading "Preventing Coagulation of the Blood" the calcium value obtained for the ostrich has already been referred to.

Magnesium, sodium and potassium values from other birds were not available, but it is interesting to note that the potassium content of ostrich blood is high when compared with that of the blood of some mammals. Groenewald (1935) recorded an average figure of 58·7 mgm. potassium per 100 c.c. bovine blood, and the following—given by Dukes (1934)—are potassium values per 1000 parts by weight of blood: Cow 0·407, Sheep 0·405, Goat 0·396.

Total Blood Volume.

For determining the approximate amount of blood in the ostrich five adult birds were used. Narcosis was induced by the administration of chloroform of which 60-80 c.c. were poured on cotton wool placed in a jar. After the right jugular vein had been severed, bleeding continued for about 45 minutes before death resulted.

The blood remaining in the heart and large vessels—about 500 c.c.—was also collected.

The average total blood volume was 5,466 c.c. and its weight constituted 5·8 per cent. of the average body weight: (Table 30).

Barlow and Biskund (1928), cited by Dukes (1937), found the weight of the blood volume of pigeons to be 6·5 per cent of the body weight.

Table 30.

Total Blood Volume.

Bird No. and Sex.	Age.	Live Weight in Kilograms.	Weight of Blood in Grams.	Calculated Blood Volume in Cubic Centimetres (Specific Gravity, 1·063).	Weight of Blood expressed as Percentage of Body Weight.
3 M.	33 Months	111·659	5,901	5,551	5·2
4 M.	3 Years	108·936	5,787	5,444	5·3
23 M.	Over 3 Years	84·879	5,787	5,444	6·8
5 F.	33 Months	108·936	6,355	5,978	5·8
24 F.	Over 3 years	82·156	5,220	4,911	6·3
Average		99·313	5,810	5,466	5·8

GENERAL DISCUSSION.

The erythrocyte count of the ostrich appears to be the lowest of the counts recorded for birds and mammals, but the ostrich erythrocyte is exceptionally large and, according to available results, it is exceeded in size only by that of the emu (*Casuarius emu*). Ponder (1919) remarks: " In general the larger the bird the larger the cell." In the moist state the cells are larger than in the dried state.

Although horse blood contains four times as many erythrocytes per c.mm. as ostrich blood, the relative volume of plasma and corpuscles of horse blood is considerably less than that of ostrich blood, and the haemoglobin content of ostrich blood is more than that of horse blood. The ostrich erythrocyte, therefore, is about four times the size of that of the horse and contains about four times as much haemoglobin. It weighs about four times as much as that of the horse, for the specific gravity of ostrich blood is about the same as that of horse blood. The points of minimum and maximum resistance of the ostrich erythrocyte are about the same as those of the erythrocyte of the fowl. The erythrocyte count of the fowl is much higher than that of the ostrich, but the haemoglobin content of fowl blood is much lower.

It is of particular interest that regeneration of erythrocytes is most clearly marked in ostrich blood. The percentage which in Romanowsky stained preparations show polychromasia is considerably higher than that observed in the blood of other animals. In vitally stained smears many reticulocytes are seen, and it is significant that in normal ostrich blood even basophil erythroblasts may constitute a fairly high percentage of the erythrocyte count.

Contrary to what would be expected, the blood of the group of unthrifty ostrich chicks, which showed marked oligocythemia as the result of severe worm-infestation, contained very few immature erythrocytes. Apparently the marrow exhausted by the long continued attempt to keep the blood up to normal eventually failed. The erythrocyte counts of the blood of the normal ostriches are fairly uniform. The coefficient of variability and the standard error of the mean can be regarded as low, but the range of 1,653,000 to 2,266,000 erythrocytes per c.mm. for normal birds is rather wide. Counts reported for the fowl show greater variation.

The leucocyte and the thrombocyte counts show considerable variation and the coefficient of variability of the thrombocyte counts is exceptionally high. Wide divergence is found also in the leucocyte and the thrombocyte counts, reported for the fowl; this may probably be largely attributed to differences in technique. It is difficult to compare results, as there is no uniform method of making total leucocyte counts of avian blood. A large number of average leucocyte counts reported for the fowl closely agree with the average count obtained for the ostrich, but the average thrombocyte count obtained for the ostrich is much lower than most counts reported for the fowl and other birds.

It is noteworthy that most of the erythrocyte and the leucocyte counts from the group of clinically healthy, semi-wild ostriches reared in the Bredasdorp District were appreciably lower than those from the five normal birds reared at Mariendahl, Stellenbosch District; also that the lymphocyte counts from the " Bredasdorp " birds were much lower than those from the " Mariendahl " birds, and the heterophile counts from the former much higher than those from the latter. As previously stated, all the " Bredasdorp " birds were probably worm-infested, but their blood gave no indication of unusual erythropoiesis, and it, therefore, appears that environmental and genetic factors might also have been partly responsible for the differences.

The percentage of lymphocytes in ostrich blood is much lower than that recorded for the fowl by most investigators, but the heterophiles constitute a much higher percentage of the leucocyte count than they do in fowl blood. On the whole, the percentages recorded for the other types of leucocytes in fowl blood do not differ greatly from those obtained for the ostrich, in which the heterophile counts showed the least variation and the eosinophile counts the greatest. The basophile of the ostrich is distinct from that of the fowl.

The inorganic phosphorus and calcium values for the group of birds grazing on natural pasture in the Bredasdorp District compare very favourably with the results from the normal birds which received a liberal supply of bones and other foodstuffs. As previously stated, the inorganic phosphorus values shown by the normal birds are suggestive of a seasonal variation.

The prolonged coagulation time of ostrich blood, which apparently cannot be associated with a low calcium content, the higher percentage volume readings obtained with old samples of ostrich blood and the marked tendency of the leucocytes to clump immediately after the blood is drawn are interesting features which offer fertile ground for further investigation.

The writer hopes that this work, which may be defined as a preliminary study of the blood of the ostrich, will provide a basis for future research.

SUMMARY.

1. Methods are described for collecting blood and preparing smears from the ostrich.

2. Ostrich blood usually has a very prolonged coagulation time and often it fails to coagulate if drawn directly from a blood vessel without coming in contact with the tissues. Heparin (1 mgm. to 5 c.c. blood) invariably prevented coagulation and it had no deleterious effect on the blood.

3. Various methods recommended for counting leucocytes in avian blood were tried on ostrich ~~blood.~~ Wiseman's method was found to be the best.

4. Morphological and biochemical studies of the blood of twenty-two ostriches were made, and the following are the average values and ranges obtained for the blood of normal ostriches:—

Erythrocyte count per c.mm. = 1,894,000 ± 14,939. Range = 1,653,000 to 2,266,000.

Relative volume of corpuscles and of plasma = 48·9 ± 0·34. Range = 40·6 to 54·0.

Minimum resistance point of red cells in sodium chloride solution = 0·47. Range = 0·44 to 0·52.

Maximum resistance point of red cells in sodium chloride solution = 0·27. Range = 0·3 to 0·25.

Sodium chloride solution which is isotonic for the erythrocytes = 0·9 per cent.

Haemoglobin content per 100 c.c. blood = 16·0 gms. Range = 14·6 to 17·2 gms.

Leucocyte count per c.mm. = 21,050 ± 419. Range = 12,326 to 32,273.

Lymphocyte count = 26·8 ± 0·5 per cent. Range = 19·2 to 37·0 per cent.

Monocyte count = 3·0 ± 0·3 per cent. Range = 0·5 to 8·5 per cent.

Heterophile count = 59·1 ± 0·9 per cent. Range = 40·5 to 78·5 per cent.

Eosinophile count = 6·3 ± 0·5 per cent. Range = 0 to 19·5 per cent.

Basophile count = 4·7 ± 0·3 per cent. Range = 1·0 to 10·5 per cent.

Thrombocyte count per c.mm. = 10,521 ± 427. Range = 2,478 to 20,086.

Viscosity: Blood = 4·5. Range = 4·0 to 5·0. Plasma 1·6. Range = 1·5 to 1·8.

Specific gravity: Blood = 1·063. Range = 1·060 to 1·065
Plasma 1·022 Range = 1·021 to 1·024

Inorganic phosphorus content per 100 c.c. blood = 9·1 ± 0·3 mgm. Range = 5·8 to 11·4 mgm.

Calcium content per 100 c.c. = 10·1 ± 0·4 mgm. Range = 8·1 to 14·2 mgm.

Magnesium content per 100 c.c. = 7·6 ± 0·4 mgm. Range = 7·1 to 8·4 mgm.

Sodium content per 100 c.c. = 273·7 ± 13·0 mgm. Range = 250·0 to 322·5 mgm.

Potassium content per 100 c.c. = 196·6 ± 6·0 mgm. Range = 177·5 to 211·5 mgm.

5. The average total blood volume was 5,466 c.c. and its weight expressed as a percentage of the average body weight was 5·8. The total blood volume ranged from 5,220 to 6,355 c.c. and its weight constituted 5·2 to 6·3 per cent. of the body weight.

6. Cell types corresponding to those seen in fowl blood occur in ostrich blood. These have been described.

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